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Population Ecotoxicology Of The Common Snapping Turtle In Agriculturally Impacted Lotic Ecosystems

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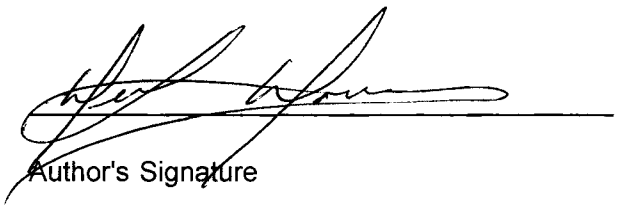

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Population Ecotoxicology of the Common Snapping Turtle in Agriculturally Impacted Lotic Ecosystems

BY

Derrick L. Douros

THESIS

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ABSTRACT

Anthropogenic impacts on lotic systems, including pollutants, have had both direct and indirect negative effects on the aquatic habitats of the world. Goals of this study are: (1) Quantify atrazine, the atrazine metabolite diaminochlorotriazine (DACT), and glyphosate burdens in common snapping turtle (*Chelydra serpentina)* tissue from individuals collected within the Embarras River in Coles County, Illinois. (2) Quantify the atrazine, DACT, and glyphosate loads in water from the aquatic habitats in which common snapping turtles reside. (3) Quantify the relative abundance of all turtle species and movement patterns of common snapping turtles in this lotic ecosystem.

Hoop nets and modified minnow traps were deployed and all turtles captured were identified to the species level. In addition, common snapping turtles were weighed, sexed, marked, and a tissue sample collected from the tail.

Concentrations of atrazine, DACT, and glyphosate in tissue did not show any relationship with distance from the spillway (Lake Charleston, Illinois), carapace length, width, or mass. Year and gender did show a trend for atrazine in the tissue. Turtles captured the second time tended to have more atrazine and glyphosate in their tissue than when they were first captured; however, a paired t-test did not reveal any significance. Both atrazine and glyphosate tissue samples varied as a function of site, but DACT did not. Tissue concentration of atrazine tended to vary with tissue concentrations of DACT. Beginning at the spillway, atrazine and glyphosate concentrations in water samples showed a linear effect on distance and a deviation from linearity. DACT water samples did not show a linear effect on distance, but there was a deviation from linearity. Water column concentrations of all three contaminants varied across capture sites, but atrazine

water concentration did not have an effect on DACT water concentration nor did it exhibit an interaction with site. Water column concentrations of atrazine and glyphosate were greater than tissue concentrations. Water and tissue concentrations of DACT did not differ. Six different species of turtles were found to occur within the river basin. Abundance for both years in descending order was the eastern painted (*Chrysemys picta*), red-eared slider (*Trachemys scripta*), eastern spiny soft-shell (*Apalone spinifera*), northern map (*Graptemys geographica*), and the eastern musk (*Sternotherus odoratus*). The common snapping turtle was the third most abundant turtle found in the river. Thus, herbivorous turtles were the most abundant and carnivorous turtles were the least abundant. Recapture data revealed that there was no effect of habitat (river vs. slough), year (2008 vs. 2009) or directionality (upstream versus downstream) on mean movement distance of common snapping turtles.

DEDICATION

This thesis is dedicated to my parents, Peter and Janet, who throughout my life have allowed and encouraged me to follow my own heart and mind, but especially to my wife, Linda, for her love, help, support, and devotion while I completed this thesis research.

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I would like to thank the members of my thesis committee, Dr. James M. Novak, Dr. Karen F. Gaines, and Dr. Stephen J. Mullin for their advice, help, and support in guiding me through this research project. The Illinois Department of Natural Resources (IDNR), the Graduate School at Eastern Illinois University, and Eastern Illinois University who provided the funding for my research project. I would like to thank the City of Charleston, Illinois, and the landowners that gave me permission to access their property for sample and data collection. Samples and data were collected under authorization of IDNR Scientific Collection Permits #NH08.5194 and #NH09.5194. Animal collection and handling was done under the authorization of Eastern Illinois University's Institutional Animal Care and Use Committee Protocol # 08-008. I would also like to thank B. Bluett, P. Brewer, D. Brown, R. Jansen, B. Jones, M. Mounce, and Dr. E. Tuttle for the use of equipment and supplies. Additionally I would like to thank the people that helped with sample collection in the field and sample processing in the laboratory K. Baumgartner, C. Bobryk, A. Datt, P. Edwards, D. Hiatt, H. Kaur, D. Kumar, J. Laird, and, P. Salvadori.

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INTRODUCTION

The anthropogenic control of lotic systems, including the effects of pollutants, has created both direct and indirect effects on the aquatic habitats of the world. Specifically, modifications of rivers and streams, along with the use of pesticides, have caused declines in the reptile species that depend on these habitats (Gibbons *et al.*, 2000). Pimentel *et al.* (1991) estimated that more than 600 types of pesticides are used in the U.S., with approximately 500,000 tons being applied every year. Pesticides are commonly used because with every dollar spent on pesticides, there is about a four-dollar return in saved crops (Pimentel *et al.*, 1992). Many pesticides ultimately end up in surface waters and may stay there for extended periods of time (Pimentel *et al.*, 1992). Two commonly used pesticides in agriculture are glyphosate, N-(phosphonomethyl)glycine, also known as Roundup® or Rodeo®, and atrazine, 2-chloro-4(ethylamine)-6-(isopropylamine)-s-triazine. Glyphosate is a non-selective broad-spectrum post-emergent herbicide, and atrazine is a pre-emergent herbicide used for broadleaf and grassy weeds (USEPA, 2008a; 2008b). According to the United States Environmental Protection Agency's 2000-2001 Pesticide Market Estimates, 39 to 41 million kg of glyphosate were applied and 34 to 36 million kg of atrazine were applied making them the most commonly used pesticides in the United States (USEPA, 2001). The USEPA has set drinking water standards of 700 parts per billion (ppb) for glyphosate and 3 ppb for atrazine (USEPA, 2009). DACT, the most important metabolic, and physical, breakdown product of atrazine, is listed on the Contaminant Candidate List 2 (USEPA, 2005). Thus, it is important to monitor DACT when assessing contaminant loads of atrazine in biotic systems (Barr *et al.*, 2007).

In fish, specifically *Prochilodus lineatus* and *Clarias gariepinus*, glyphosate has been found to cause histological abnormalities as indicated by increased plasma glucose levels, and physiological changes by such as an increase in catalase liver activity and several other irregularities associated with improper liver function (Langiano and Martinez, 2008; Olurin *et al.*, 2006). Glyphosate has also been shown to stop steroidgenesis because it disrupts expression of the steroidgenic acute regulatory protein (Walsh *et al.*, 2000). Dallegrave (2007) found that when male Wistar rats (*Rattus norvegicus*) were exposed to glyphosate *in utero* and during lactation they showed a decrease in testosterone levels and low sperm counts.

At doses well below EPA drinking water standards (3 ppb), atrazine exposure in larval American Leopard Frogs (*Rana pipiens*) caused reduced germ cells, gonadal dysgenesis, and hermaphroditism (Hayes *et al.*, 2003). Sprague-Dawley rats (*Rattus norvegicus*) showed a decrease in testosterone, prostate mass, and seminal vesicle size when exposed to atrazine (at or above 100 ppb) (Trentacoste *et al.*, 2001). Likewise, Nieves-Puigdoller *et al.* (2007) determined that Atlantic salmon (*Salmo salar*) smolts exposed to atrazine had disrupted endocrine systems, growth problems, ionoregulatory anomalies, decreased gonadosomatic index (males) and a marked increase in hepatosomatic index (females) at concentrations of 100 $\mu g \cdot L^{-1}$ (100 ppb). In these studies, it is unclear if the observed effects are the result of exposure to atrazine or one of its metabolites. In a temperature controlled laboratory experiment, Willingham (2005) found that red-eared slider (*Trachemys scripta elegans)* hatchlings from eggs dosed with atrazine (0.5 ppb) in the low temperature range were as large as the hatchlings from eggs

incubated at the mid-range temperature. Furthermore, the number of female hatchlings was greater when atrazine was present at increased temperatures, thus indicating an interaction of atrazine and temperature on sex-determination in this turtle species. Common snapping turtle eggs incubated in atrazine treated soil (at concerntrations of either 1.479 kg/ha or 14.79 kg/ha) did not show a difference in the number of intersex or female hatchlings produced when compared to eggs incubated in the absence of atrazine (De Solla *et al.,* 2006).

The use of herbicides has created both direct and indirect effects on the aquatic systems of the world. One previously undiscovered direct effect is the global decline in the number of amphibians and reptiles (Gibbons *et al.*, 2000). Relative to the number of described species, reptiles are in greater jeopardy of extinction compared to amphibians worldwide, with turtles being at greatest risk (Gibbons *et al.*, 2000). Several reptile species have been the subject of toxicological studies. These studies have determined the effects of contaminants but, the methods and tissue types analyzed are usually species specific, and therefore few general conclusions can be drawn. In addition, reptiles are underrepresented in toxicological studies (Hall, 1980, 1992; Hopkins, 2000).

One reptile species that has been the focus of a number of toxicological studies is the common snapping turtle (*Chelydra serpentina*). Snapping turtles have temperature-dependent sex determination (Janzen, 2008), and therefore toxicological studies have been performed on the eggs (Gibbons *et al.,* 2000; De Solla *et al.*, 2006). The snapping turtle exhibits many characteristics that facilitate their use as a representative reptile for toxicological studies: (1) A fossil record dating back to the Pliocene, about 3 to 5 million years ago (Hutchison, 2008). Turtles as a group date back about 250 million years, and

have shown relatively little change in morphology in the last 200 million years (Gilbert, 1993). (2) Snapping turtles only exist in North America, with a native range east of the Rocky Mountains in the U. S. and the southern tip of Canada (Ernst and Barbour, 1972). This area contains approximately 137,588,700 ha of land on which the primary land use is high intensity agriculture. (3) Snapping turtles live in aquatic systems that are nutrient and herbicide sinks from agricultural practices. (4) They are a long-lived species and can grow to over 34 kg with a carapace length of more than 47 cm (Ernst and Barbour, 1972). This allows for exposure to be monitored over longer time periods. (5) They are opportunistic omnivores and will eat insects, birds, small mammals, fish, molluscs, crustaceans, and plant matter (Pell, 1940). Consequently, snapping turtles provide an integrated snapshot of contaminant levels across trophic levels. Although many life history, demographic and movement studies have been performed on snapping turtle populations, few have been carried out in riverine systems (Steyermark *et al.*, 2008).

The purpose of this study is to determine if common snapping turtles can be utilized as sentinel species for the detection of agriculture herbicides within lotic ecosystems. If so, an additional purpose is to determine how their movement patterns and associated land-uses affect toxicant burdens. Therefore, the specific objectives of this study are to: (1) Quantify atrazine, the atrazine metabolite diaminochlorotriazine (DACT), and glyphosate body burdens in common snapping turtle tissue from individuals collected from the Embarras River in Coles County, Illinois. (2) Quantify the atrazine, DACT, and glyphosate loads in water from an aquatic habitat in which common snapping turtles reside. (3) Quantify the relative abundance of turtles and movement patterns of snapping turtles in this lotic ecosystem.

Based on these objectives, the working hypotheses are: (1) The snapping turtle will accumulate atrazine and/or DACT over its lifetime. (2) The snapping turtle will accumulate glyphosate over its lifetime. (3) Concentrations of atrazine, DACT, and glyphosate are higher in the snapping turtle compared to concentrations found in the water where they were trapped. (4) Relative abundance of herbivorous turtles will be greater than the relative abundance of omnivorous turtles, which will be greater than the abundance of carnivorous turtles in the Embarras watershed. (5) Movement patterns of the snapping turtle will be different in running water compared to still waters (slough, oxbow and backwater regions) of a riverine system.

This study will provide a further characterization of snapping turtle ecology and toxicology and broaden the characterization of riverine turtle populations. It will also assess the feasibility of using the snapping turtle as a sentinel species for anthropogenic impacts in riverine systems. This study also has many broader impacts. It will increase our knowledge on movement patterns of the common snapping turtle in riverine systems where few studies exist. The use of ELISA methodology for determining herbicide concentrations in tissue samples will allow for less costly estimation, and thus broader investigation, of contaminant body burdens. Because they live in riverine ecosystems, and in many cases the same systems used to supply human drinking water, long-term exposure and bioaccumulation of contaminants in the snapping turtle may be translated to human risk from these contaminants. For this paper the current EPA definition of bioaccumulation will be used. Bioaccumulation is a "general term describing a process by which chemicals are taken up by an organism either directly from exposure to a contaminated medium or by consumption of food containing the chemical" (USEPA,

2010a). Finally, because turtle populations are declining, this study can be used by managers for the conservation of not only snapping turtles, but riverine turtles as a group, including those turtles that are currently threatened or endangered, such as the smooth softshell turtle (*Apalone mutica*) which occurs in the Embarras River (Pers. Obs.).

MATERIALS and METHODS

STUDY SITE:

The Embarras River originates just south of Champaign-Urbana in east-central Illinois. It flows southeasterly and joins with the Wabash River near Lawrenceville, Illinois, about 312 river km from its origin. The northern portion of the river basin was formed during the Wisconsin Episode of the Quaternary ice age about 15,000 to 20,000 years ago; the southern portion of the river basin was formed during the Illinois Episode of the Pleistocene between 300,000 to 130,000 years ago. The Embarras River basin drains an area of about 6320 km^2 (Wiggers, 1998). In 1947, the City of Charleston, Illinois, erected Riverview Dam east of Illinois Route 130, which formed Lake Charleston. The dam is a low-head dam that acts as a spillway when water levels are at or above normal levels. The "lake" is approximately 62 ha and served as the city's water supply until 1981, when the City of Charleston built the Charleston Side Channel Reservoir next to Lake Charleston. Water is pumped from Lake Charleston to the Charleston Side Channel Reservoir, which is the City of Charleston's sole source of drinking water (IEPA, 1996). The river was sampled from the dam and upstream approximately 9.1 river km (Figure 1).

No values for Total Maximum Daily Load (TMDL) exist for either atrazine or glyphosate in the Embarras watershed. Only phosphorous loads are controlled via a

TMDL. The watershed has existing impairments for total phosphorous, fecal coliform, dissolved oxygen, total suspended solids, total nitrogen, manganese, sediment/siltation, nitrogen, pH, and unknown cause. No historic data for atrazine or glyphosate concentrations in water or biota could be found for the Embarras watershed (USEPA, 2010b).

TURTLE DATA:

Sampling:

Hoop nets were deployed and baited with frozen carp pieces contained within a small metal cage (Lagler, 1943a). In 2008, there were 7 sites, and in 2009 there were 9 sites, with each site having 10 hoop nets. The nets were left at each site for 4 days and the bait was changed after the second day. Trapping started in July of 2008 due to record flooding in that year (USGS, 2010), and in May of 2009. Trapping ceased in late October in both years after two consecutive bouts of trapping with no snapping turtle captures. The traps were set where water levels are conducive to capturing turtles as described by Froese (1978). The location of the nets was recorded using a hand-held Garmin GPSmap 76CSx global positioning system. Modified metal minnow traps were deployed at all net locations for capturing juvenile turtles. All turtles captured were identified to the species level, counted, and after processing, released where captured. Because snapping turtles were marked but other species were not, counts for snapping turtles include both captures and recaptures to make them comparable to the counts in other species. Each captured snapping turtle was individually marked using a battery-operated drill to create holes in the marginal scutes. The holes were placed in accordance to a marking system described by Cagle (1939). The mass (± 10 g), and carapace length

and width (± 1 cm), of each snapping turtle was recorded. The gender of each snapping turtle was determined by rocking the turtle on a raised platform to elicit the eversion of the cloaca. Any individual that did not exhibit an everted cloaca was categorized as an unknown.

The tail of each turtle was washed with deionized water and 2.5% lidocaine solution was administered topically using an atomizing sprayer. A 5 to 10 mm tail snip was taken using aseptic clippers and the tissue was placed in a sterile vial. The vial was placed in ice until returned to the laboratory and then stored at -6 °C until the tissue sample could be processed. To determine glyphosate and atrazine accumulation over the snapping turtle's lifetime, size (mass, carapace width and/or carapace length) was used as a surrogate measure of age. Classification of an herbivorous, omnivorous, and carnivorous diet for purposes of relative abundance was determined by a literature review of the life history of each species (Lagler, 1943b). Turtle trapping and tissue collection procedures were performed according to Eastern Illinois University IACUC protocol # 08-008 and IDNR scientific collection permit numbers NH08.5194 and NH09.5194.

Laboratory Analysis:

Tissue samples were thawed and chopped into small pieces using a sterile razor blade. The sample was then homogenized using a Fisher Scientific Power Gen cryogenic homogenizer. Approximately 7 ml of Fisher Scientific brand HPLC grade methanol was added to each sample and sonicated for 6 min using a Branson Sonifier 250 with the microtip output set at 3 and the duty cycle adjusted to 55% (Scutaru *et al.*, 1988). The samples were then centrifuged for 20 min using a Fisher, centrific™ centrifuge model 225 (Impens *et al.*, 2003; Scutaru *et al.*, 1988). The supernatant was removed and

evaporated at ambient temperature under an enclosed and darkened hood. Double deionized water (1.5 ml) was added to each sample vial, and the vials were allowed to equilibrate overnight at room temperature. The sample was then extracted from the vial and filtered using a Puradisc™ 25 AS disposable syringe filter device (Whatman, Inc.). Each sample was analyzed to determine concentrations of atrazine, DACT, and glyphosate using a competitive para-magnetic enzyme linked immunosorbent assay (ELISA) kit. The kits used were the Atrazine HS (high sensitivity) Assay Kit (Product No. 500007), the Glyphosate Assay Kit (Product No. 500081), and the Triazine Metabolite ELISA (Microtiter Plate) (DACT) Assay Kit (Product No. 520006), all obtained from Abraxis LLC, (Warminster, PA). Analyses were conducted using the instructions enclosed in each kit. For the glyphosate assay the alternative derivatization procedure was used. These procedures can be found at the Abraxis web site (www.abraxiskits.com). In addition to a positive control that is included in each kit, a spike was prepared and analyzed with each run. For the glyphosate kit, a spike was prepared at a concentration of 2.0 ppb using a standard prepared by SPEXCertiPrep (Lot Number T1091009026; 2,000 ppb). For the atrazine kits a spike of 2.0 ppb was prepared using 98.9% pure atrazine purchased from Chem Service, Inc. (West Chester, PA). The atrazine samples were run using 30% replication, glyphosate and DACT samples were run using 100% replication.

Statistical Analysis:

Tissue concentrations of atrazine, DACT, and glyphosate were assessed using multiple linear regression models of contaminant body burden with turtle mass, carapace length, carapace width, and the distance from the spillway as independent variables. In addition, tissue concentrations of atrazine, DACT, and glyphosate were analyzed with ANOVA using year, site, capture/recapture and subject gender as independent variables. A paired t-test was also used to test if contaminant tissue concentrations were different between turtles captured in multiple years. The effect of month of capture was analyzed using ANOVA. In addition, the months were collapsed into season to reflect differences in herbicide application rates in May, June and July compared to August, September and October. A log-linear categorical model was utilized to test for differences in abundances of the three trophic groups – herbivores, omnivores, and carnivores. Mean distance moved was analyzed as a function of habitat (riverine versus slough) and direction of movement (upstream versus downstream) using one-way ANOVA. A model selection approach was used, based upon Akaike Information Criterion (AIC), for any general or generalized linear models with the overall goal of identifying the simplest model that adequately explained the data (Burnham and Anderson, 2002). SAS© v 9.2 was used for all data analysis. An alpha-level of 0.05 was used to determine statistical significance. All means are reported as Mean Value ± 95% confidence limits.

WATER DATA:

Sampling :

Water samples were taken at every turtle hoop net location in 2008 and at every fifth turtle hoop net location in 2009 (70 samples in 2008 and 18 in 2009). All water samples

were placed in 0.5-L opaque plastic bottles. Water samples were collected from the first 10 cm of the water column. The samples were brought back to the laboratory and stored at 9 °C until analyzed.

Laboratory Analysis:

Water samples were filtered using vacuum pump filtration with a non-binding glass microfiber type GF/C filter (Whatman, Inc.) in a Büchner funnel to remove suspended sediment. Water samples were analyzed to determine concentrations of atrazine, glyphosate, and DACT using a competitive para-magnetic enzyme linked immunosorbent assay (ELISA) kit. The kits used were Atrazine HS (high sensitivity) Assay Kit 100T (Product No. 500007), Glyphosate Assay Kit 120T (Product No. 500081), and Triazine Metabolite ELISA (Microtiter Plate) (DACT) Assay Kit (Product No. 520006), all obtained from Abraxis LLC, (Warminster, PA). Analyses were conducted using the procedure outlined in each kit. For the glyphosate assay the alternative derivatization procedure was used. The specific procedures can be found at the Abraxis web site (www.abraxiskits.com). In addition to a positive control that is included in each kit, a spike was prepared and analyzed with each run. For the glyphosate kit the spike was prepared at a concentration of 2.0 ppb using a standard prepared by SPEXCertiPrep (Lot Number T1091009026; 2,000 ppb). For the atrazine kits a spike of 2.0 ppb was prepared using 98.9% pure atrazine purchased from Chem Service, Inc. (West Chester, PA). The double deionized water used for dilutions had a concentration of 0.00 ppb for all contaminants. The atrazine samples were analyzed using 30% replication; glyphosate and DACT samples were analyzed using 100% replication.

Statistical Analysis:

Concentrations of atrazine, DACT, and glyphosate in the water samples were assessed using simple linear regression models of contaminant concentrations with distance from the spillway as the independent variable. In addition, deviation from linearity was also assessed (Zar, 1999). For models exhibiting non-linearity, hierarchical polynomial regression models (Zar, 1999) of distance were fit to the data. The best fit model was determined with a model selection procedure based upon the AIC (Burnham and Anderson, 2002). Water column concentrations of atrazine, DACT, and glyphosate were also analyzed using ANOVA with site as an independent variable. In addition, a heterogeneity of slopes model was run for DACT using atrazine as the covariate. SAS© v 9.2 was used for all data analysis. An alpha-level of 0.05 was used to determine statistical significance. All means are reported as Mean Value ± 95% confidence limits.

RESULTS

Tissue concentrations of atrazine, DACT and glyphosate, respectively, showed no relationship with distance from the dam (for each contaminant, respectively: $t_{85} = -0.09$, $P = 0.93$; $t_{84} = -0.88$, $P = 0.38$; $t_{85} = 1.34$, $P = 0.18$; Figures 2, 3, 4, 5 and 6), turtle carapace length ($t_{85} = 1.16$, $P = 0.25$; $t_{84} = 0.70$, $P = 0.48$; $t_{85} = 0.41$, $P = 0.68$), turtle carapace width ($t_{85} = -0.60$, $P = 0.55$; $t_{84} = -1.29$, $P = 0.20$; $t_{85} = -0.43$, $P = 0.67$) or turtle mass ($t_{85} = 0.16$, $P = 0.87$; $t_{84} = 0.34$, $P = 0.73$; $t_{85} = -0.08$, $P = 0.93$; Figures 7, 8, 9). In addition, tissue concentrations of DACT showed no relationship to tissue concentrations of atrazine ($t_{84} = -0.22$, $P = 0.83$). The multiple regression models did not explain much of the variation (Atrazine: $R^2 = 0.06$, $df_{Model} = 4$, $df_{Error} = 85$; DACT: $R^2 = 0.03$, $df_{Model} = 5$, $df_{Error} = 84$; Glyphosate: $R^2 = 0.03$, $df_{Model} = 4$, $df_{Error} = 85$).

Atrazine and glyphosate concentration in turtle tissues varied as a function of site (atrazine: $F_{40,52} = 7.93$, $P = 1.13 \times 10^{-11}$, $R^2 = 0.86$; glyphosate: $F_{40,49} = 39.49$, $P = 3.20 \times 10^{-26}$, $R^2 = 0.97$). However, there was no effect of site on the tissue concentration of DACT ($F_{40,50} = 0.67$, $P = 0.90$, $R^2 = 0.35$). Neither atrazine nor DACT concentrations in the water samples had an effect on the tissue concentration of DACT (water atrazine: $F_{1,49} = 0.01$, $P = 0.93$; water DACT: $F_{1,49} = 0.02$, $P = 0.89$). In this ANCOVA model, however, the concentration of DACT in the tissue tended to vary with changes in the concentration of atrazine in the tissue ($F_{1,49} = 3.13$, $P = 0.08$).

Tissue concentrations of atrazine also tended to vary by study year (2009 > 2008; $F_{1,90} = 3.38$, $P = 0.07$) and subject gender (Male > Unknown; $F_{1,90} = 3.47$, $P = 0.07$; Figure 2), but not as a function of the interaction between year and gender ($F_{1,89} = 1.40$, $P = 0.24$). Neither year nor gender exhibited main or interaction effects with either DACT (Year: $F_{1,88} = 1.18$, $P = 0.28$; Gender: $F_{1,88} = 0.74$, $P = 0.39$; Interaction: $F_{1,87} = 0.07$, $P = 0.79$; Figure 3) or glyphosate (Year: $F_{1,87} = 2.46$, $P = 0.12$; Gender: $F_{1,87} = 0.74$, $P = 0.39$; Interaction: $F_{1,86} = 0.23$, $P = 0.63$; Figure 6). Whereas carapace length did not differ between genders ($F_{1,93} = 1.96$, $P = 0.17$, $r^2 = 0.02$), males tended to have larger carapace widths compared to unknowns ($F_{1,93} = 3.62$, $P = 0.06$, $r^2 = 0.04$) and larger values for length corrected mass (ANCOVA; Gender: $F_{1,93} = 5.67$, $P = 0.02$, Length: $F_{1,93} = 242.45$, $P = 1.2 \times 10^{-27}$, $R^2 = 0.74$). Turtles captured a second time had higher levels of atrazine ($F_{1,91} = 5.25$, $P = 0.02$) and glyphosate ($F_{1,88} = 4.82$, $P = 0.03$) but not DACT ($F_{1,89} = 0.50$, $P = 0.48$) in their tissue. Paired t-tests did not reveal any differences between turtles caught for the first and second time for tissue concentrations of atrazine ($t_{14} = 1.59$, $P = 0.14$), DACT ($t_{14} = 0.45$, $P = 0.66$) or glyphosate ($t_{14} = 1.45$, $P = 0.17$) (Figure 10).

Lastly, Paired t-tests did not reveal any differences between mean tissue concentrations of trapping sites that were trapped in both 2008 and 2009 for atrazine ($t_{13} = 1.20$, $P = 0.25$), DACT ($t_{13} = -0.42$, $P = 0.68$) or glyphosate ($t_{13} = 1.55$, $P = 0.15$).

Tissue concentrations for atrazine tended to vary as a function of the month of capture ($F_{4,90} = 2.08$, $P = 0.09$), but neither DACT ($F_{4,87} = 0.30$, $P = 0.89$) nor glyphosate ($F_{4,87} = 0.95$, $P = 0.44$) exhibited monthly variation (Figure 11). Analyzing temporal differences between the early trapping months (May, June and July: Higher) in comparison to the later trapping months (August, September and October: Lower) revealed differences for atrazine ($F_{1,93} = 7.11$, $P = 0.01$) and glyphosate ($F_{1,90} = 3.74$, $P = 0.06$) but not for DACT ($F_{1,90} = 0.09$, $P = 0.77$) (Figure 12).

Atrazine concentrations in water samples showed a complex pattern of variation with distance up-river from the dam (Lake Charleston). There was a linear effect of distance from the dam on atrazine concentration ($F_{1,224} = 22.51$, $P = 3.72 \times 10^{-6}$). However, there was also a deviation from linearity ($F_{64,160} = 2.57$, $P = 8.79 \times 10^{-7}$). A quintic model provided the best polynomial fit (AICc = -770.71, evidence ratio = 2.71 over next best model; Figures 13, 15, and 16). DACT concentration did not exhibit a linear effect of distance from the dam ($F_{1,262} = 0.093$, $P = 0.76$). There was, however, a deviation from linearity ($F_{9,253} = 14.584$, $P = 5.61 \times 10^{-19}$). A sextic model provided the best polynomial fit (AICc = -851.01, evidence ratio = 821.80 over next best model; Figures 14, 15 and 16). Glyphosate concentrations in water samples exhibited both a linear effect of distance from the dam ($F_{1,206} = 26.201$, $P = 7.04 \times 10^{-7}$) and a deviation from linearity ($F_{23,183} = 8.8394$, $P = 1.68 \times 10^{-19}$). A quintic model provided the best polynomial fit (AICc = -567.04, evidence ratio = 2.17 over next best model; Figure 17).

Atrazine concentration in water samples varied by site ($F_{68,223} = 2.88$, $P = 2.35 \times 10^{-9}$, $R^2 = 0.56$). There was no interaction of atrazine and site ($F_{10,59} = 1.17$, $P = 0.34$) nor a main effect of atrazine ($F_{1,59} = 0.01$, $P = 0.93$) but there was a main effect of site ($F_{10,263} = 13.14$, $P = 1.27 \times 10^{-18}$, $R^2 = 0.34$) on DACT. Glyphosate concentration also varied as a function of site ($F_{28,207} = 12.51$, $P = 4.59 \times 10^{-31}$, $R^2 = 0.66$).

Paired t-tests for each collection site indicated that atrazine concentrations in water samples exceeded those in turtle tissue ($t_{94} = 14.87$, $P = 1.99 \times 10^{-26}$). DACT concentrations in tissue did not differ from water concentrations ($t_{91} = 1.39$, $P = 0.17$). Glyphosate concentrations in water exceeded tissue concentrations of glyphosate ($t_{91} = 9.74$, $P = 8.92 \times 10^{-16}$) (Figure 18).

A total of 773 turtles were captured in 2008 and 2009 representing six different species. The most abundant species in 2008 and 2009 was the eastern painted turtle (*Chrysemys picta*) with a total of 415 turtles trapped (2008, 167 and 2009, 248). The second most abundant species was the red-eared slider (*Trachemys scripta*) with a total of 99 captured in 2008, and 116 captures in 2009, for a total of 215. The third most abundant species was the common snapping turtle. In 2008, 58 were trapped and in 2009, 57 were caught for a total of 115 snapping turtles captured (captures and recaptures combined). Thirty-one eastern spiny softshells (*Apalone spinifera*) were caught in 2008, and 40 were captured in 2009, for a total of 71 caught for both years. A total of 9 northern map turtles (*Graptemys geographica*) were captured with 3 caught in 2008 and 6 caught in 2009. The least abundant turtle trapped was the eastern musk turtle (*Sternotherus odoratus*). Two and three musk turtles were captured in 2008 and 2009, respectively (Figure 19). Across both study years, the number of individuals per species

differed ($G_5 = 466.29$, $P = 1.5 \times 10^{-98}$). The total number of individuals captured also differed between years, with more turtles being caught in 2009 ($G_1 = 14.49$, $P = 0.0001$). The abundance pattern of species did not differ between years ($G_5 = 5.01$, $P = 0.42$).

Trophic groups followed the expected abundance pattern. Primarily herbivorous species were most numerous, while carnivorous species were the least common (Table 1, Figure 20). Across both study years, the number of individual turtles representing each trophic group differed ($G_2 = 525.14$, $P = 9.3 \times 10^{-115}$). The total number of individuals captured also differed between years, with 2009 having the larger number of turtles captured ($G_1 = 14.49$, $P = 0.0001$). The abundance pattern of trophic levels did not differ between years ($G_2 = 2.55$, $P = 0.30$).

Recapture data revealed that there was no effect of habitat type (river channel vs. slough) on movement distance in either year ($F_{1,23} = 2.65$, $P = 0.12$). The distance moved by snapping turtles also did not differ between years in the different habitats ($F_{1,3} = 0.05$, $P = 0.83$). There was no indication that distance moved varied between habitats as a function of year ($F_{1,23} = 0.10$, $P = 0.76$). There were also no differences in the directionality of movement (upstream, vs. downstream) in either year ($F_{1,17} = 0.32$, $P = 0.59$). Likewise, directionality did not show any difference between 2008 and 2009 ($F_{1,23} = 0.02$, $P = 0.90$). There was no indication that distance moved varied between directions as a function of year ($F_{1,23} = 0.82$, $P = 0.38$; Figure 21).

DISCUSSION

When considering the concentrations of atrazine, DACT, and glyphosate in the tissue samples from collected turtles, no relationships with distance, carapace length, carapace width, and mass existed. Furthermore, these data could lead to the conclusion that bioaccumulation in the tissue has reached an equilibrium in the turtles. Size could be a surrogate for age in the same water system in a localized area. This fact along with the strong effect of site on water concentration, the inter-site variation, and the dedicated home-range of the turtles (Obbard and Brooks, 1981) may result in this being an inaccurate test for bioaccumulation. This is evidenced by the pattern of site variation in the tissue concentration for atrazine and glyphosate, but not DACT.

Atrazine concentration varied as a function of year and gender in turtle tissue but DACT and glyphosate did not. Males exhibited higher concentrations of atrazine compared to turtles of unknown gender. The larger size of male turtles is not the likely cause of this pattern as no size variable was related to tissue concentration of any contaminant. Atrazine has a relatively high water solubility and low fat solubility compared to other organic contaminants (Dobbs and Williams, 1983). Therefore, gender based differences in body mass are also unlikely to be of much import. Male turtles spend more time in the water compared to females. Females leave the river to nest and will depurate contaminants into their eggs (DeSolla and Fernie, 2004) and thus males may have an increased exposure to atrazine in the spring when females are nesting.

The year effect may indicate that bioaccumulation of atrazine had not reached an equilibrium because the mean tissue concentration in 2009 is larger than the mean tissue concentration in 2008. The mean concentration of atrazine and glyphosate in tissue

samples from recaptured turtles was higher compared to the mean of those same turtles captured the first time plus individuals sampled only once. Again, this suggests that neither atrazine nor glyphosate bioaccumulation had reached an equilibrium. Year is confounded with recapture in this test but it is not the same test, statistically. The most direct tests for nonequilibrium of bioaccumulation are those using paired t-tests for sites that yielded turtles in both years and between individual turtles trapped twice. These tests are not significant for either atrazine or glyphosate and thus indicate bioaccumulation of these two contaminants have reached equilibrium. When failing to reject the null hypothesis, however, power and sample size are of paramount importance. Both of the paired t-tests suffer from a much reduced sample size (N = 14 and 15, respectively) compared to the year and recapture tests (N= 91 and 92, respectively). Therefore, we have conflicting evidence as to whether atrazine and glyphosate in turtles have reached equilibria between uptake and depuration. Sample sizes need to be increased in terms of numbers recaptured as well as number of years sampled.

The water samples for atrazine, DACT, and glyphosate did not show any apparent change in concentration with regards to the linear distance along the Embarras River. The sample concentrations did show a complex spatial pattern and explained only a low amount of the variation. Much of the variation in the water samples for all contaminants was explained by site. The effect of site on both atrazine and glyphosate in water samples can be explained by the land-use near the site locations. The higher concentrations of contaminants are located at or near an application site and/or near the point of entry into the waterway for the chemical (Figure 22).

DACT shows no pattern with any measured variables in this study. However, the levels of DACT were stable over time, space and between water and turtle tissue. Thus, DACT appears to be in equilibrium in turtles, the water column, and between both of these system components. As a metabolic byproduct of atrazine degradation, DACT should be less sensitive to variation in herbicide application as a function of time or space, and thus, is the preferred biomarker for assessing the state of the aquatic system. However, DACT has no physiological or metabolic relationship with glyphosate concentrations. The major metabolic byproduct of glyphosate is aminomethylphosphonic acid (AMPA) (Kolpin *et al.*, 2006). Unfortunately, there is no current ELISA based method for determining AMPA concentrations. In addition, because AMPA is a necessary compound for synaptic transmission, careful control values would be necessary to detect increased equilibrium levels. This appears to be a fruitful avenue for future research.

Many factors dictate the turtle species caught within an aquatic system, including habitat availability and quality, water type, human interference, and trapping time (Conner *et al.*, 2005; Dreslik *et al.*, 2005). The results support aspects of other studies that assess the community structure of turtles, among which is that snapping turtles are usually the third- or fourth-most common species (Conner *et al.*, 2005; Dreslik *et al.*, 2005). When categorizing turtles based upon trophic groups (herbivores, omnivores, and carnivores), the relative abundance of each group, did not differ from the numerous other studies that trapped and recorded a wide range of species throughout the United States (Bodie *et al.*, 2000; Stone *et al.*, 2005). These results, do not appear to vary between lotic and lentic ecosystems.

The majority of prior studies performed on movement patterns of snapping turtles were executed in ponds or small lakes at more northerly latitudes (e.g., Obbard and Brooks, 1981) and found that snapping turtles have a well-defined home range (averaging 3.44 ± 1.55 ha). Movements of the snapping turtle as a function of gender has been studied during the activity season. Movement of males were found to peak in May and then decline, female movement peaked in June and then began declining (Brown and Brooks, 1993). Therefore, the finding of no difference in upstream, downstream, river habitat, or slough habitat in either year, or between years, is consistent with the snapping turtle having a defined home range and exhibiting relatively high site philopatry in both lotic and lentic bodies of water. The 2008 trapping season began after record flooding in east-central Illinois, including the Embarras River (USGS, 2010). This flooding was severe and persistent enough to wash adult male snapping turtles downstream (Pers. Obs.). Turtle species such as snapping turtles and sliders are capable of moving back upstream after floods (Plummer and Shirer, 1975). Eight of the 12 turtles that were trapped more than once in 2008 made directed upstream movements and 2 of those turtles moved more than 2,000 m. None of the turtles moved downstream more than 900 m even though longer movement distances were possible. These movements were likely returns to established home ranges and suggest a relatively high degree of philopatry in adult snapping turtles. The snapping turtle prefers a permanent slow-moving or still fresh water source, although it will enter into and live in brackish water (Conant and Collins, 1998; Froese, 1978). Snapping turtles favor mud substrates and areas where cover from obstructions are located, generally in the form of vegetation or fallen vegetative debris, in the water (Froese, 1978). These microhabitats might be patchily distributed, especially in

lotic aquatic systems, thus accentuating the philopatry and constricting movement patterns (Aars and Ims, 2000).

The association of turtle tissue concentrations of atrazine and glyphosate with site is consistent with the evidence for site fidelity in adult snapping turtles. The high site fidelity in adult snapping turtles makes it likely that site variation and individual variation are highly correlated. This relationship should also be revealed in greater individual variance in rates of bioaccumulation and would increase the sample size necessary to demonstrate bioaccumulation using individual based tests. The effect of year on atrazine may have more to do with the time of sampling. Because atrazine can be used as both a pre- and post-emergent herbicide (USEPA, 2008b) there was likely less atrazine applied in 2008 due to the flood-shortened growing season. Because of severe flooding in 2008, neither turtles nor water samples were obtained until late July. Thus, any pre-emergent application of atrazine in autumn of 2007 was likely washed out of the system or environmentally degraded by the time water and tissue samples were collected. In contrast, turtles were trapped from May through October in 2009, and spring flooding was at average levels. This should have increased the potential exposure of turtles to atrazine during the time of tissue collection in 2009. Both atrazine and glyphosate exhibited reduced tissue concentrations in August – October as compared to May – July, which is consistent with this hypothesis. The water concentrations of contaminants also exhibit differences between sites but with no consistent directional pattern. This indicates that the inter-site differences in tissue concentrations are primarily the result of differences in uptake from the water rather than differences in elimination rates.

The results of this research indicate that snapping turtles are appropriate sentinels for the effects of agrochemicals in aquatic ecosystems. They are especially useful for long-term monitoring because of their long life spans, site fidelity and tolerance of most environmental contaminants, at least compared to most fish and amphibians (Hall, 1980). Nevertheless, there is some evidence that reptiles in general may be declining globally (Gibbons *et al.*, 2000), so linking their use as sentinels to specific cases of decline can be useful both in terms of turtle conservation as well as for monitoring degradation of aquatic habitats.

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Table 1. Primary trophic level for all turtle species captured during the course of the study.

SPECIES	FEEDING HABIT
Eastern Painted	Herbivore
Red-Eared Slider	Herbivore
Common Map	Herbivore
Common Musk	Omnivore
Common Snapping	Omnivore
Eastern Spiny Soft-shell	Carnivore

Table 2. Model selection results of contaminant concentrations in water samples for non-linear polynomial effects of distance from the spillway. AICc is the small sample Akaike Information Criterion (Hurvich and Tsai, 1989), ΔAICc is the difference between the current model and the best-fit (Lowest AICc) model, w_i is the Akaike weight (scaled relative probability) and Evidence Ratio is the relative likelihood of the current model divided by the relative likelihood of the next best fitting model (Burnham and Anderson, 2002).

Atrazine					
Model	Number of Parameters	AICc	DAICc	w_i	Evidence Ratio
Quintic	6	-770.712	0.000	0.720	2.709
Sextic	7	-768.719	1.993	0.266	19.492
Quartic	5	-762.779	7.933	1.363E-02	13.430
Cubic	4	-757.584	13.128	1.015E-03	192.578
Quadratic	3	-747.063	23.649	5.270E-06	5.510
Linear	2	-743.650	27.062	9.565E-07	
DACT					
Sextic	7	-851.010	0.000	9.986E-01	821.802
Quintic	6	-837.587	13.423	1.215E-03	13.224
Quartic	5	-832.423	18.587	9.190E-05	3.464
Cubic	4	-829.938	21.072	2.653E-05	1.970
Linear	2	-828.582	22.428	1.347E-05	2.601
Quadratic	3	-826.670	24.340	5.177E-06	
Glyphosate					
Quintic	6	-567.035	0.000	6.816E-01	2.168
Sextic	7	-565.487	1.548	3.143E-01	130.843
Cubic	4	-555.739	11.296	2.402E-03	1.436
Quartic	5	-555.015	12.020	1.673E-03	807339.861
Quadratic	3	-527.812	39.223	2.072E-09	3.589
Linear	2	-525.256	41.779	5.772E-10	

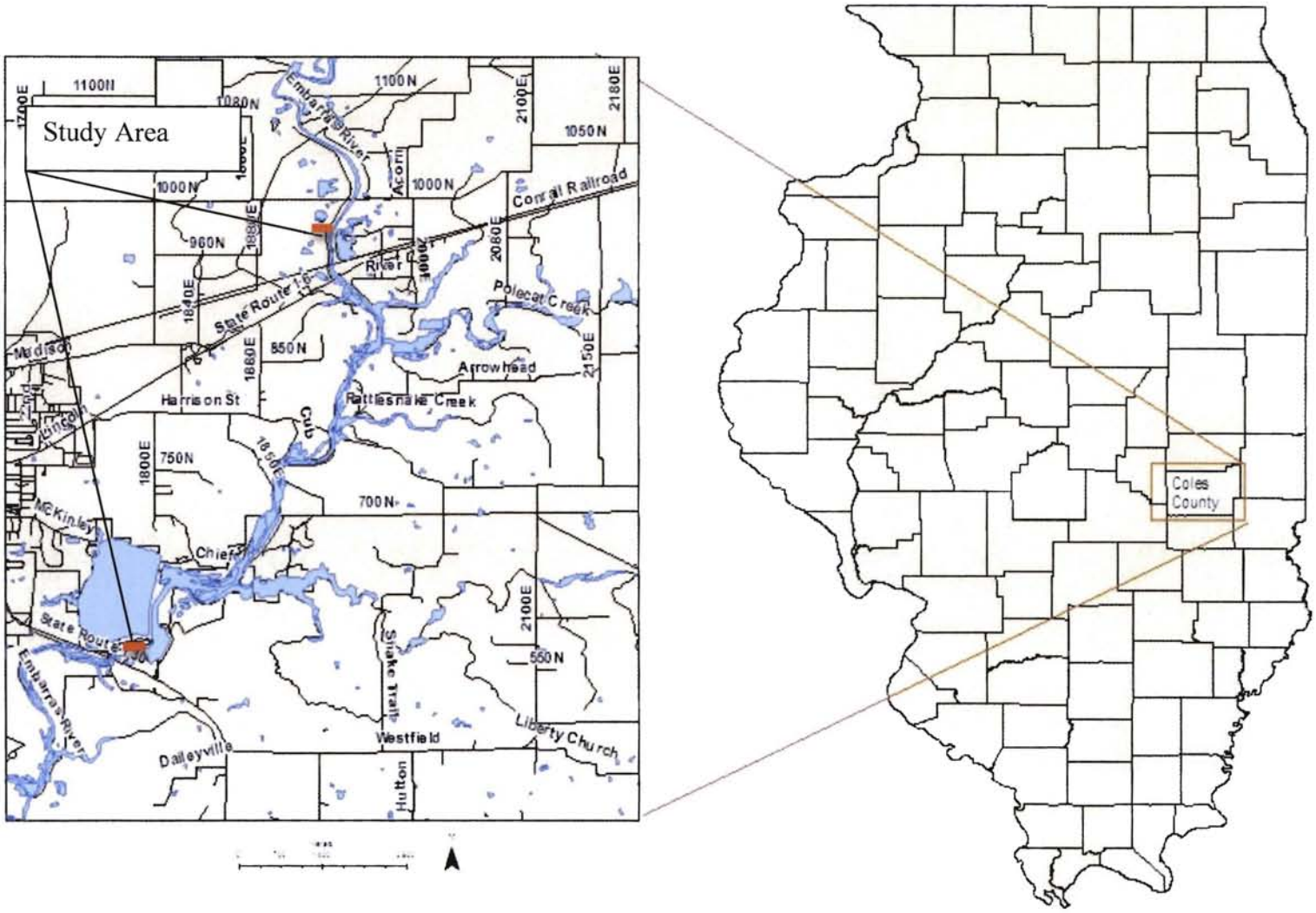

Figure 1. Section of the Embarras River watershed (Coles County, IL) sampled during 2008 and 2009.

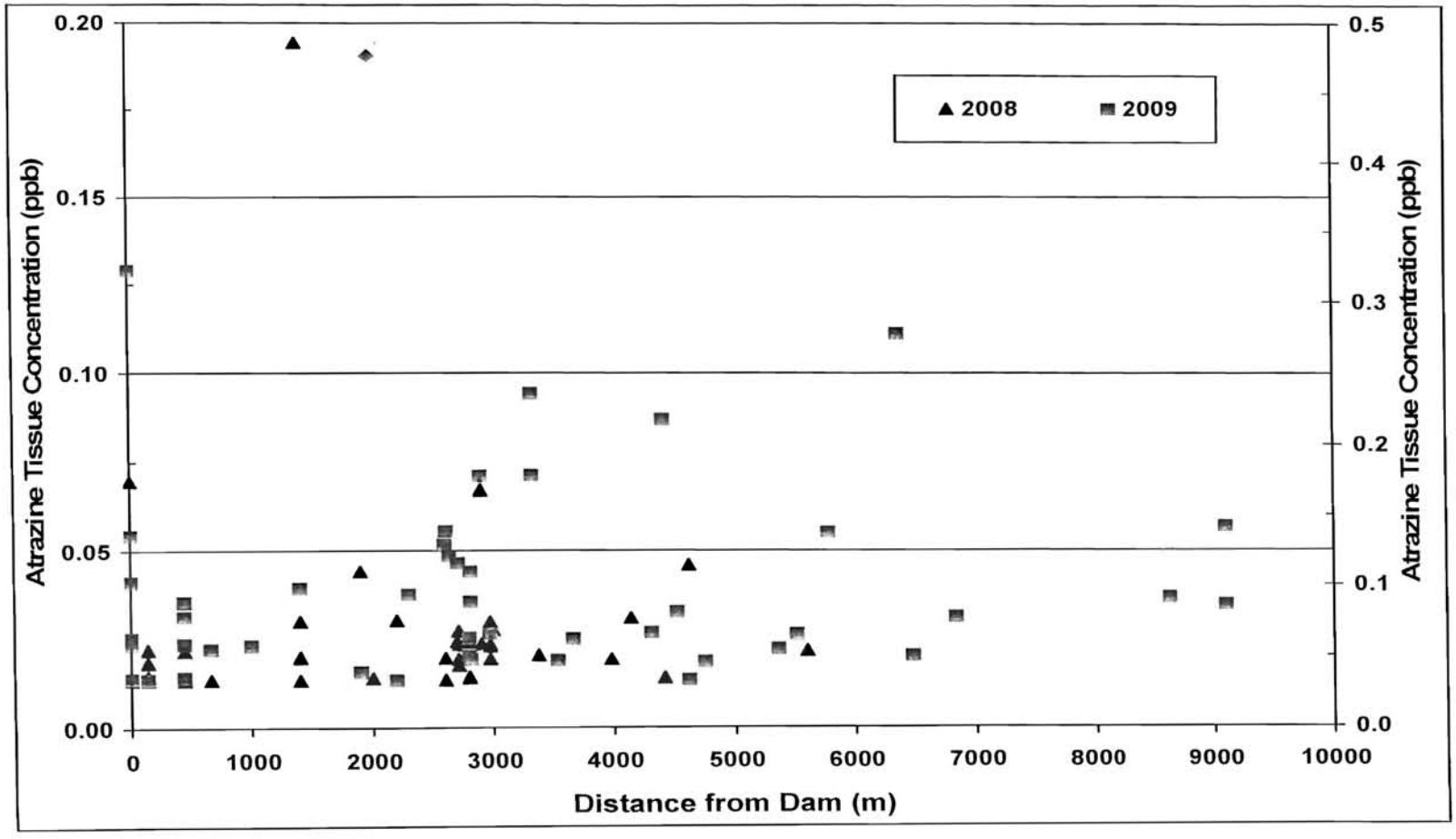

Figure 2. Concentration of atrazine found in common snapping turtle tissue in 2008 and 2009 in the Embarras River, Illinois. Values below 0.2 ppb (square/triangle) are plotted on left vertical axis; values above 0.2 ppb (diamond) are plotted on the right vertical axis.

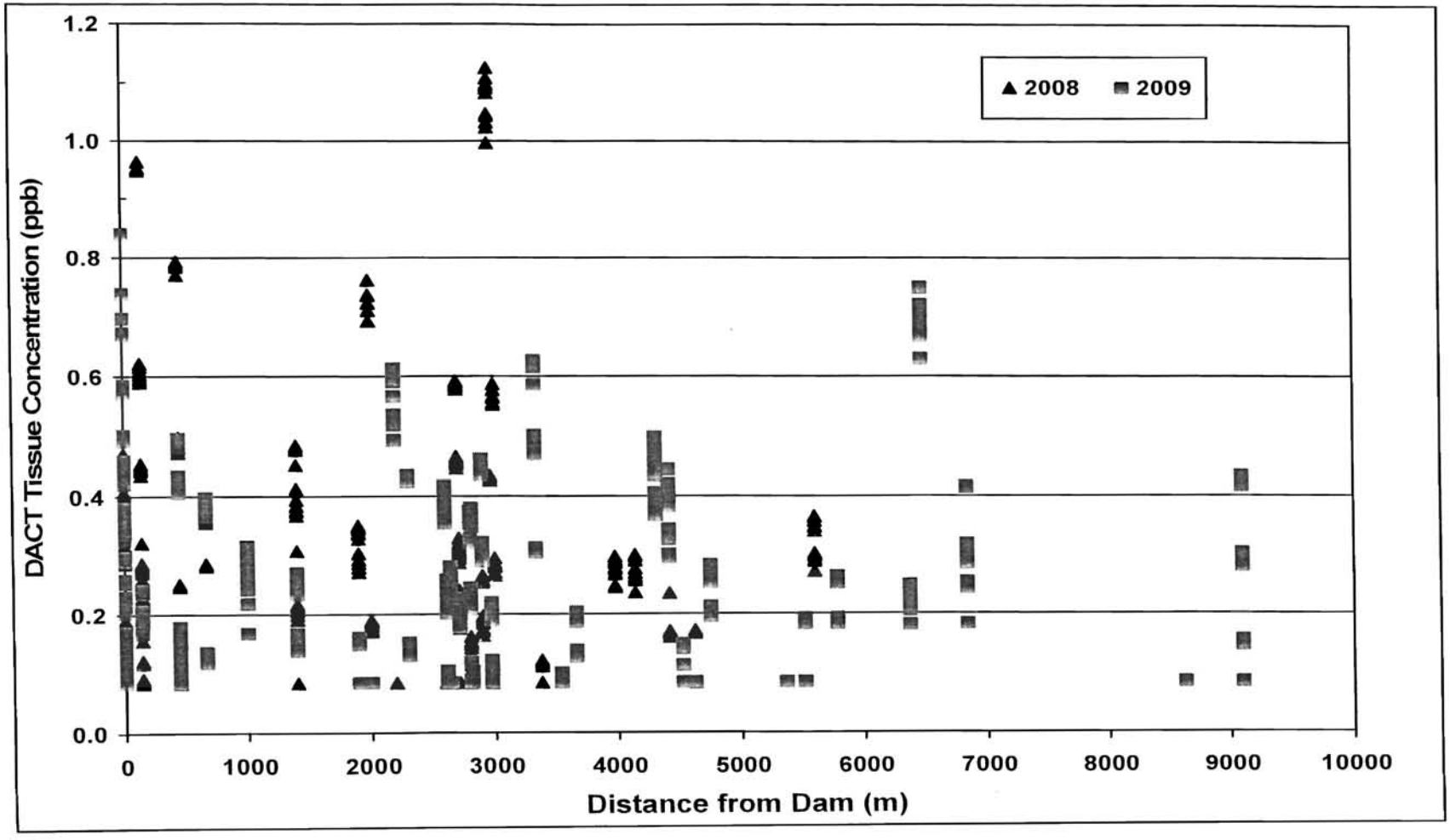

Figure 3. Concentration of diaminochlorotriazine (DACT) found in common snapping turtle tissue in 2008 and 2009 in the Embarras River, Illinois.

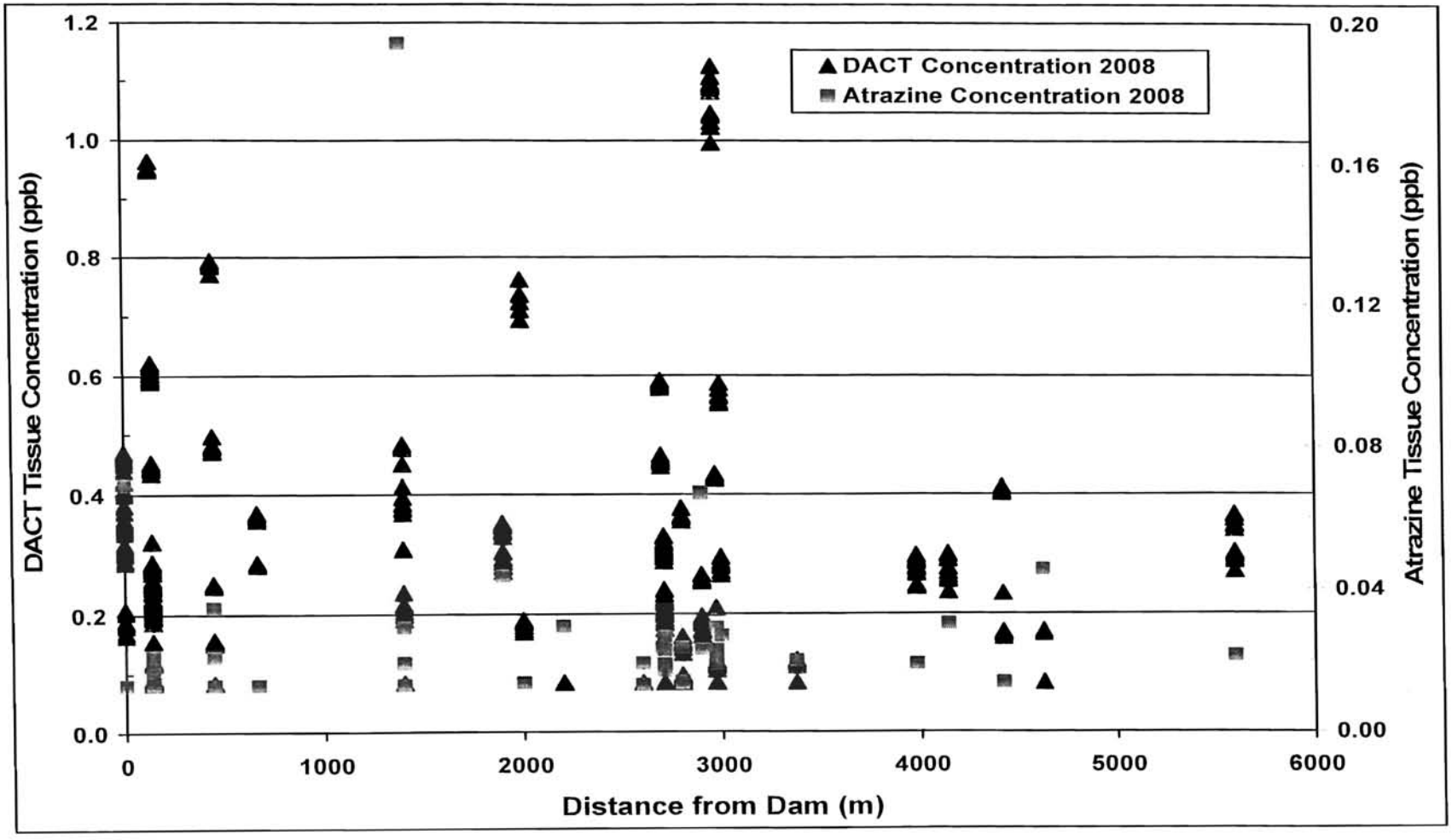

Figure 4. Concentration of DACT and atrazine found in common snapping turtle tissue in 2008 in the Embarras River, Illinois.

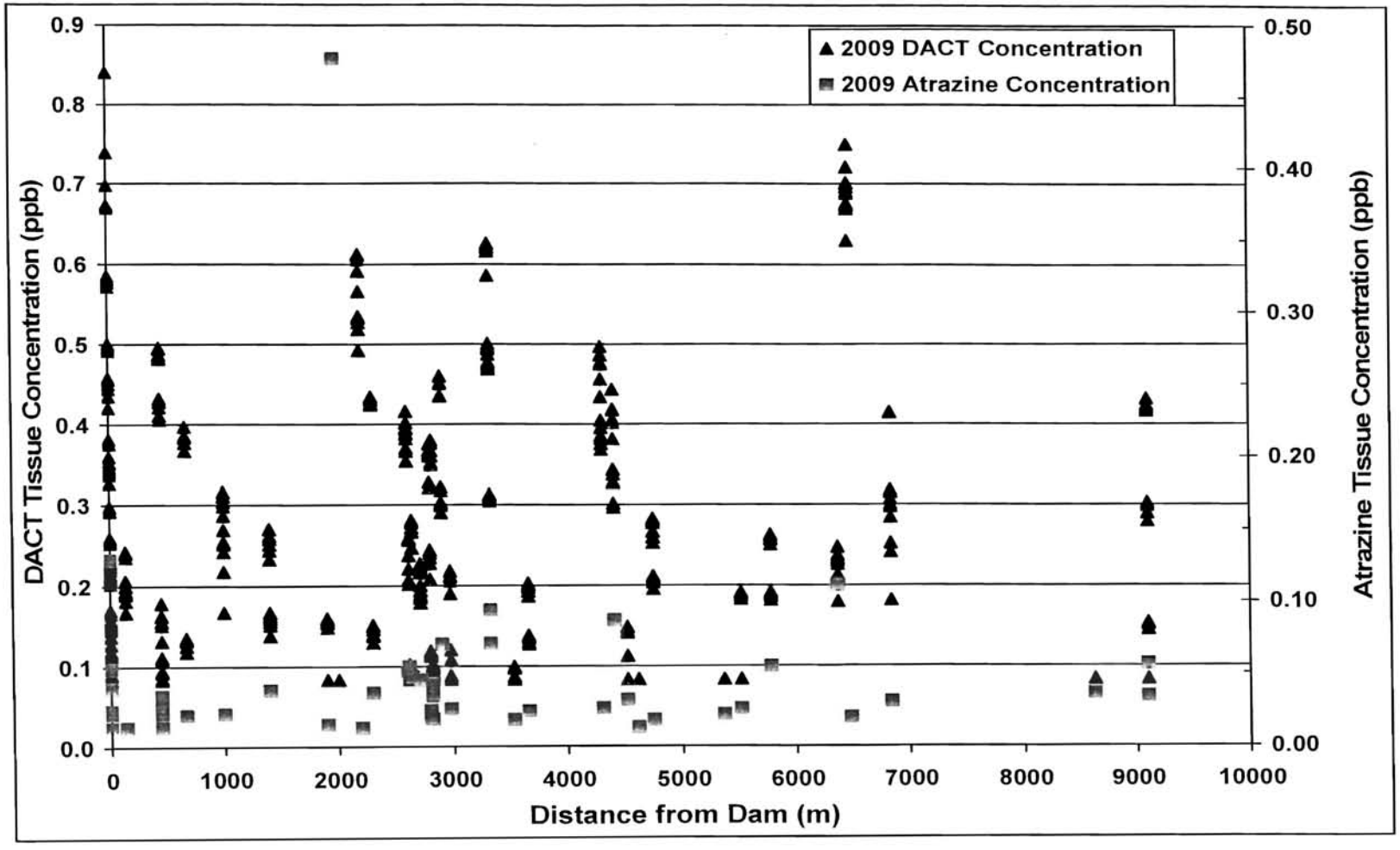

Figure 5. Concentration of DACT and atrazine found in common snapping turtle tissue in 2009 in the Embarras River, Illinois.

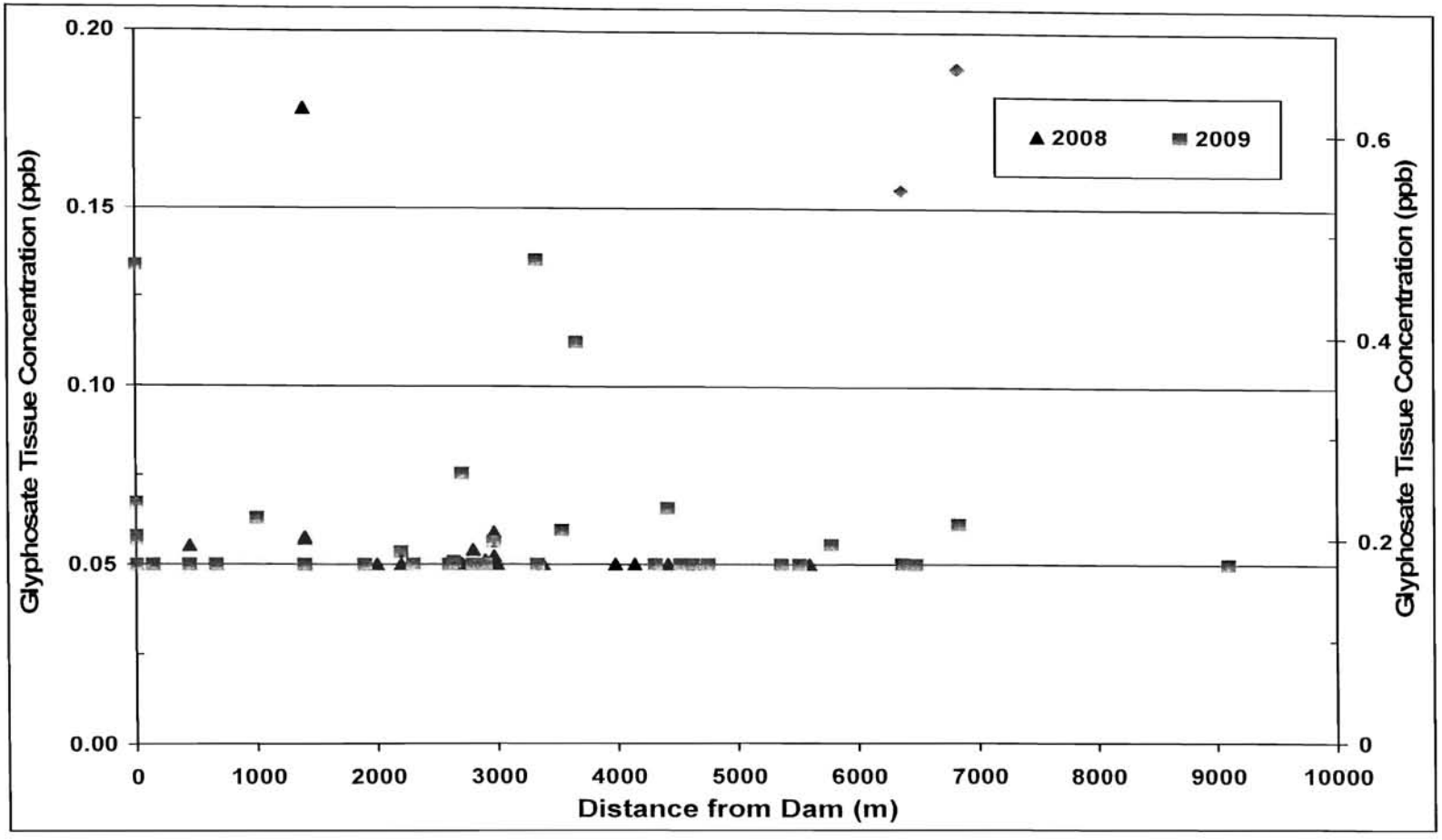

Figure 6. Concentration of glyphosate found in common snapping turtle tissue in 2008 and 2009 in the Embarras River, Illinois. Values below 0.2 ppb (square/triangle) are plotted on the left vertical axis and values above 0.2 ppb (diamond) are plotted on the right vertical axis.

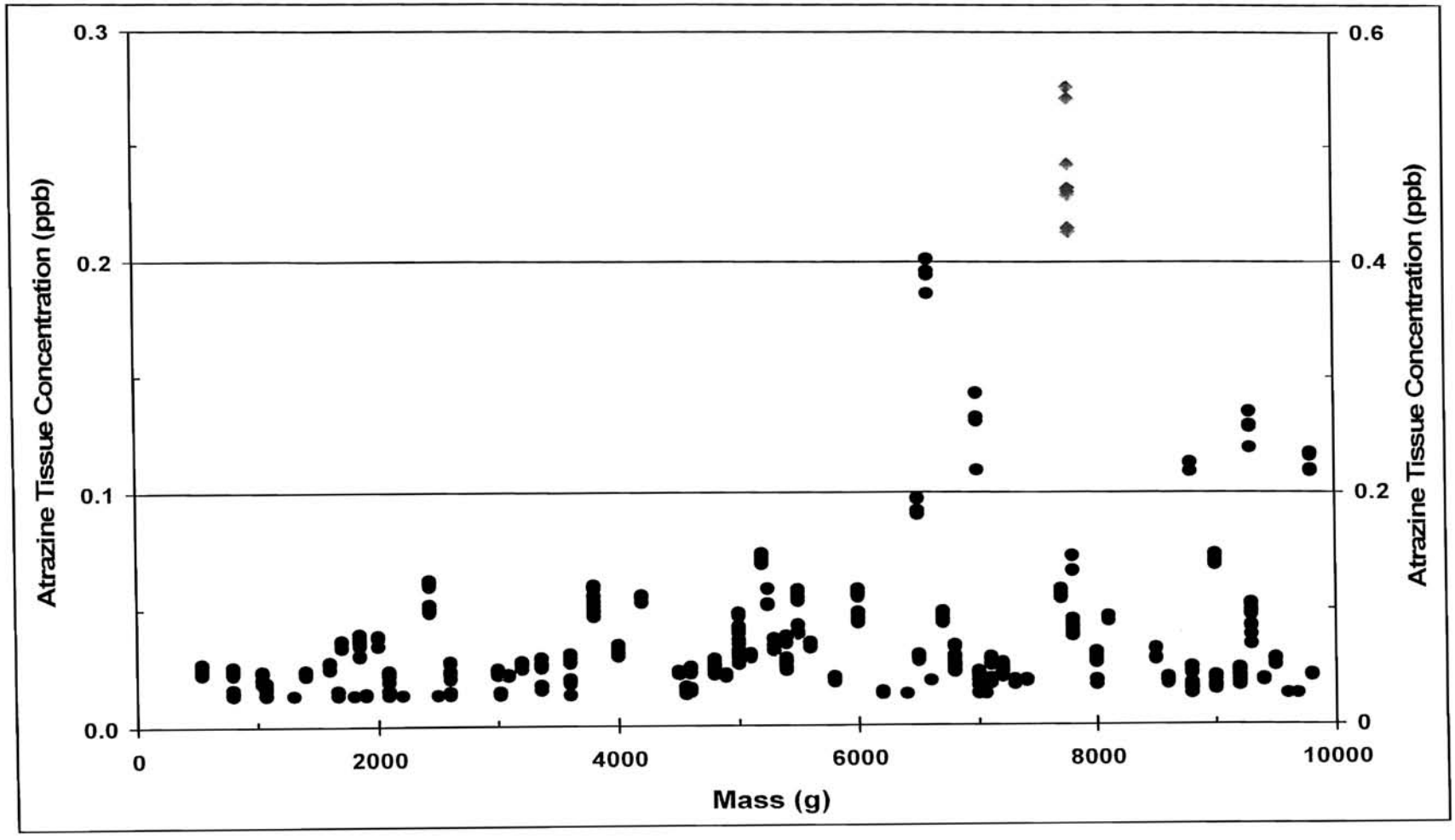

Figure 7. Atrazine concentration found in common snapping turtle tissue as a function of turtle mass. Values below 0.3 ppb (circles) are plotted on the left vertical axis, concentrations above 0.4 ppb (diamonds) are plotted on the right vertical axis.

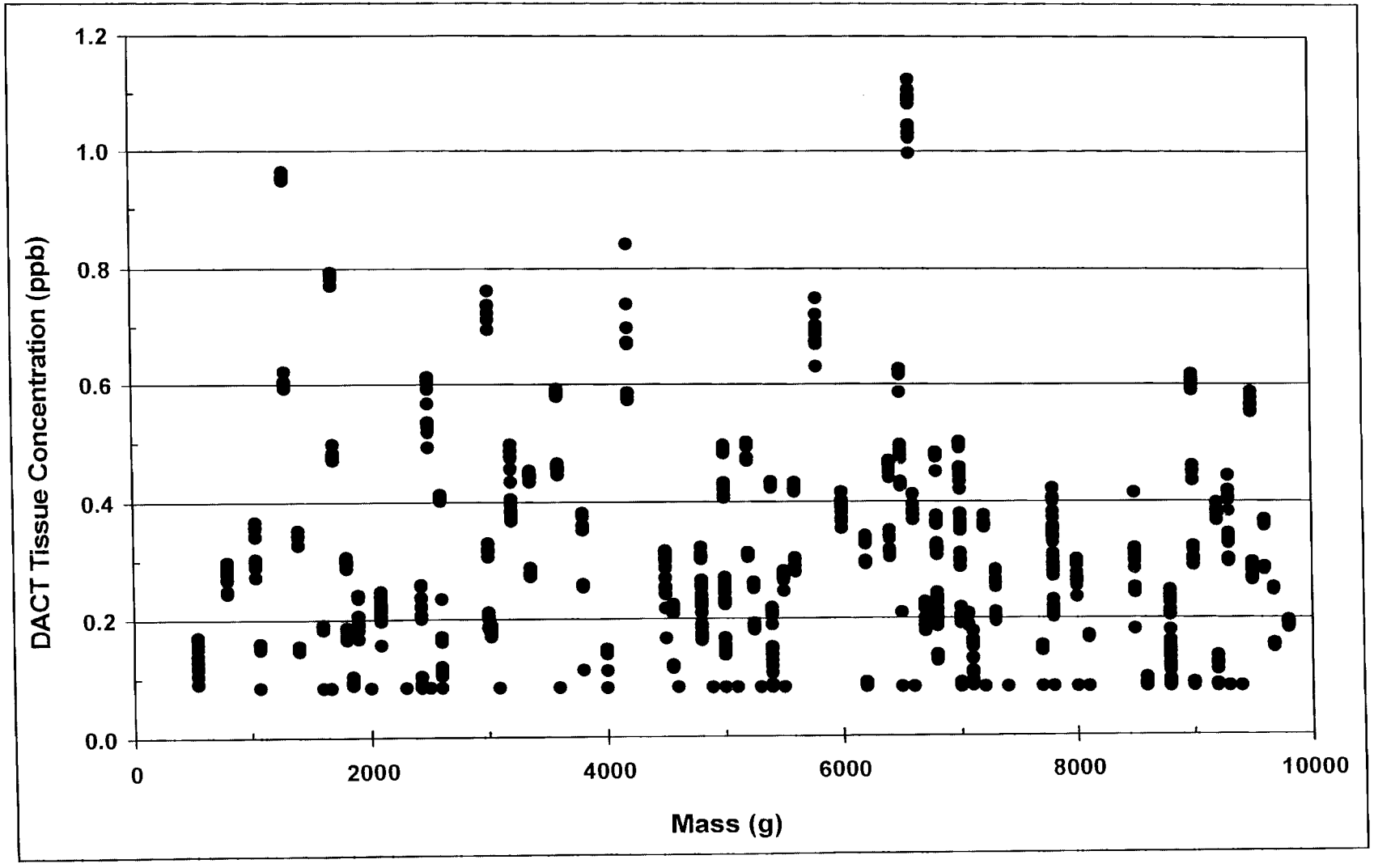

Figure 8. DACT concentration found in common snapping turtle tissue as a function of the mass of the turtle.

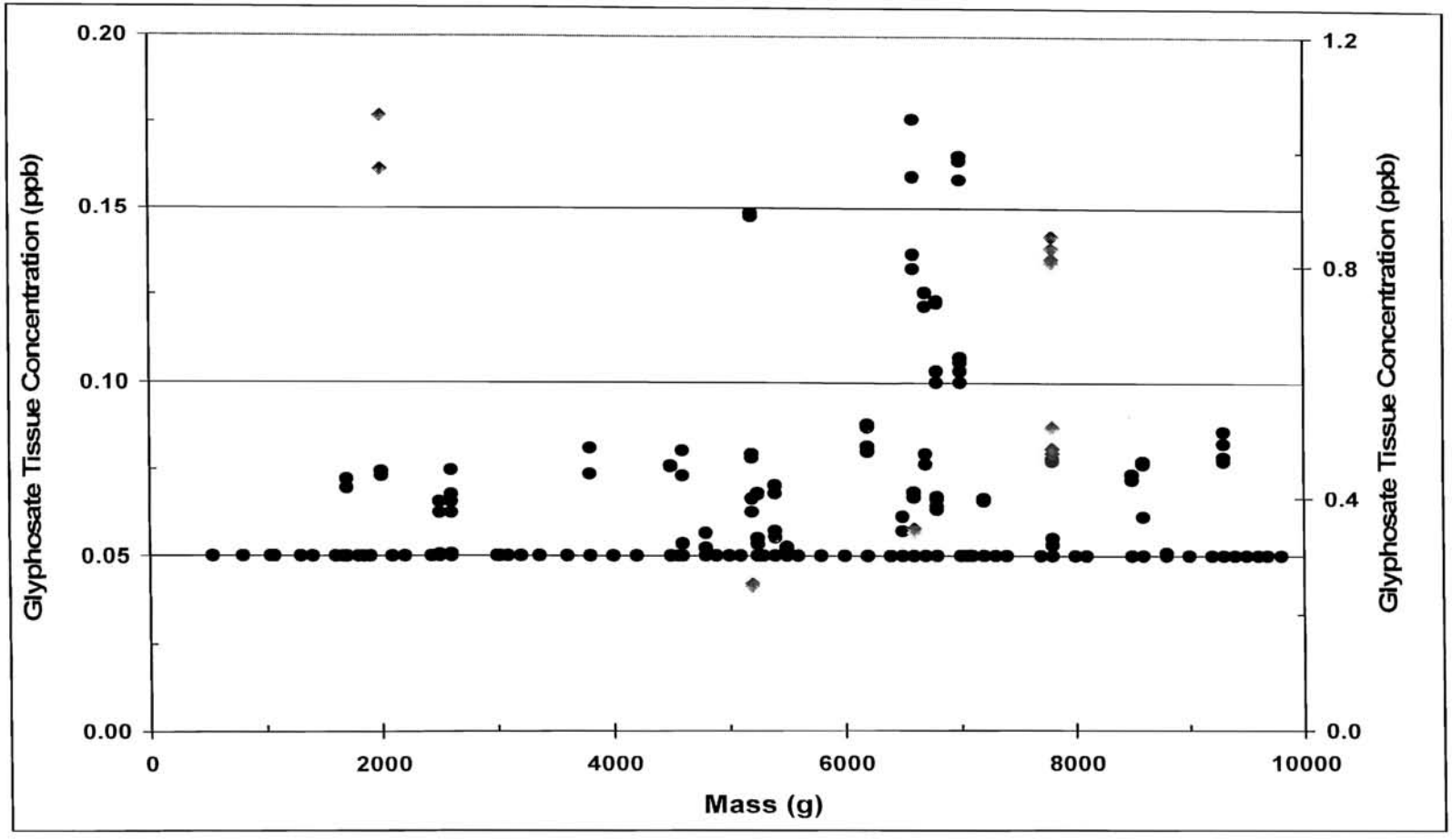

Figure 9. Glyphosate concentration found in common snapping turtle tissue as a function of the mass of the turtle, values below 0.2 ppb (circle) are plotted on the left vertical axis and values above 0.2 ppb (diamond) are plotted on the right vertical axis.

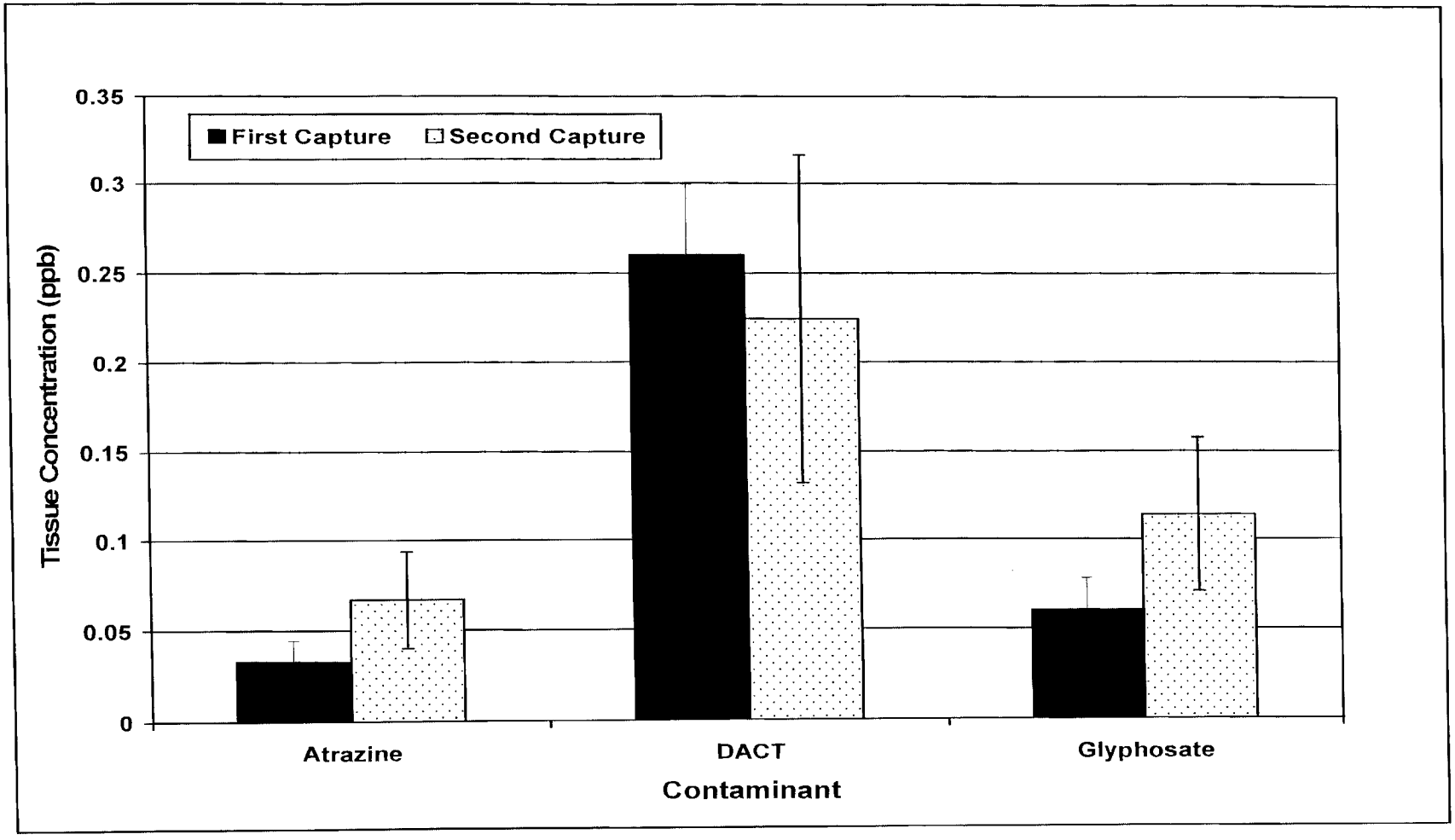

Figure 10. Mean concentrations of atrazine, DACT, and glyphosate in common snapping turtle tissue for individuals on their first capture or only captured once compared to individuals on their second capture.

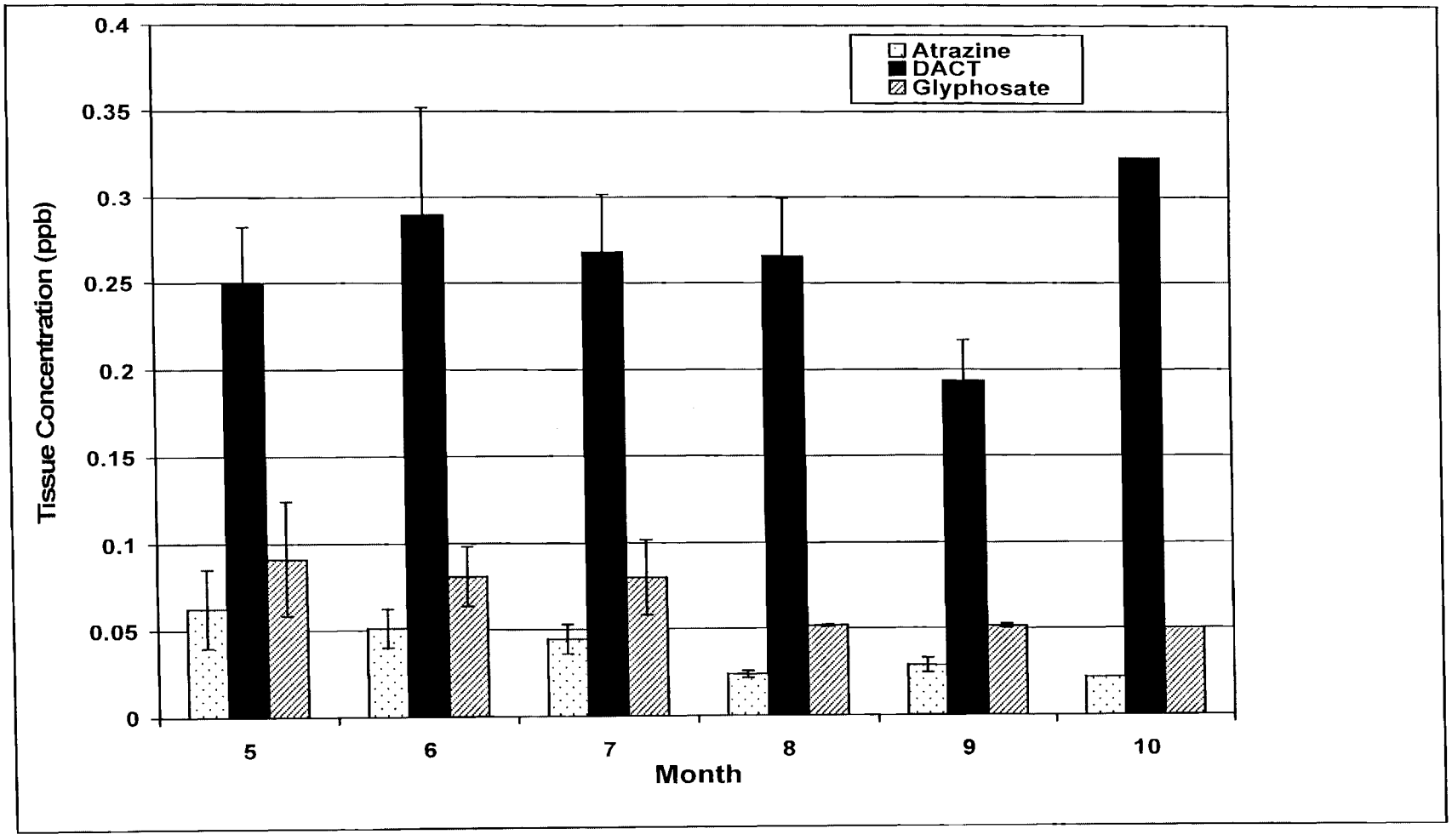

Figure 11. Mean concentrations of atrazine, DACT, and glyphosate in common snapping turtle tissue as a function of the month of capture.

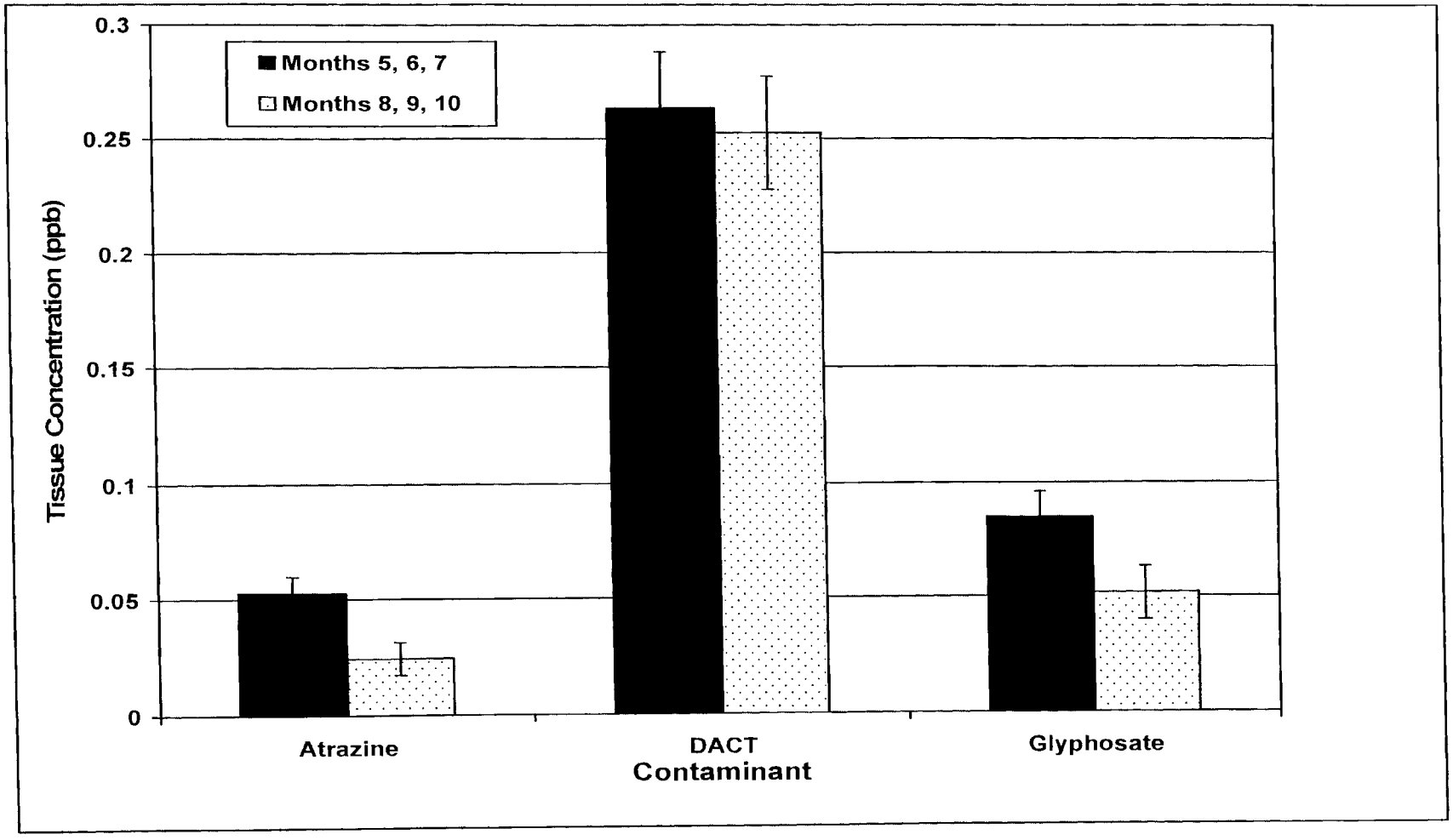

Figure 12. Mean concentration of atrazine, DACT, and glyphosate in common snapping turtle tissue as a function of the season of capture.

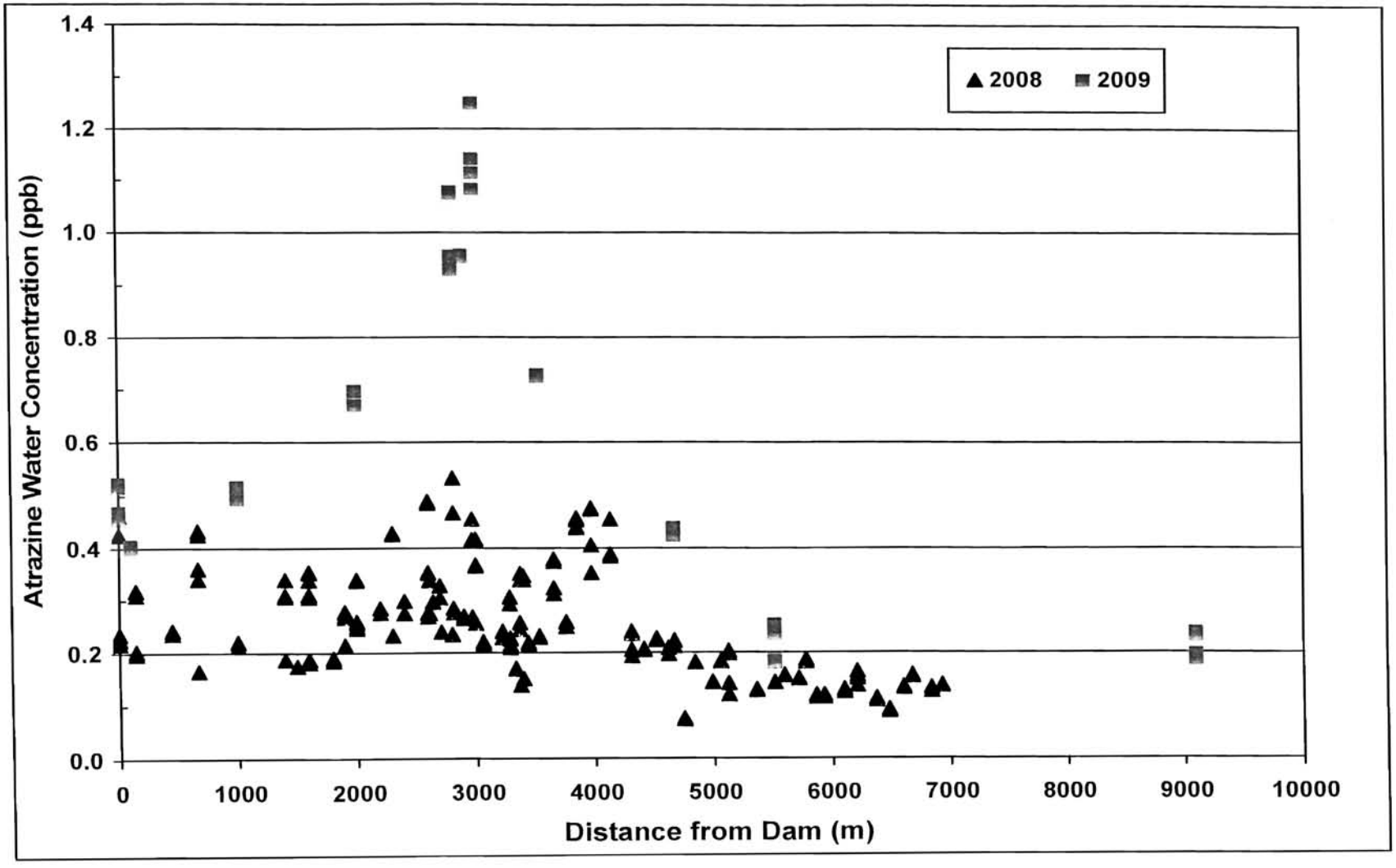

Figure 13. Concentrations of atrazine found in water samples from the Embarras River, Illinois, in 2008 and 2009.

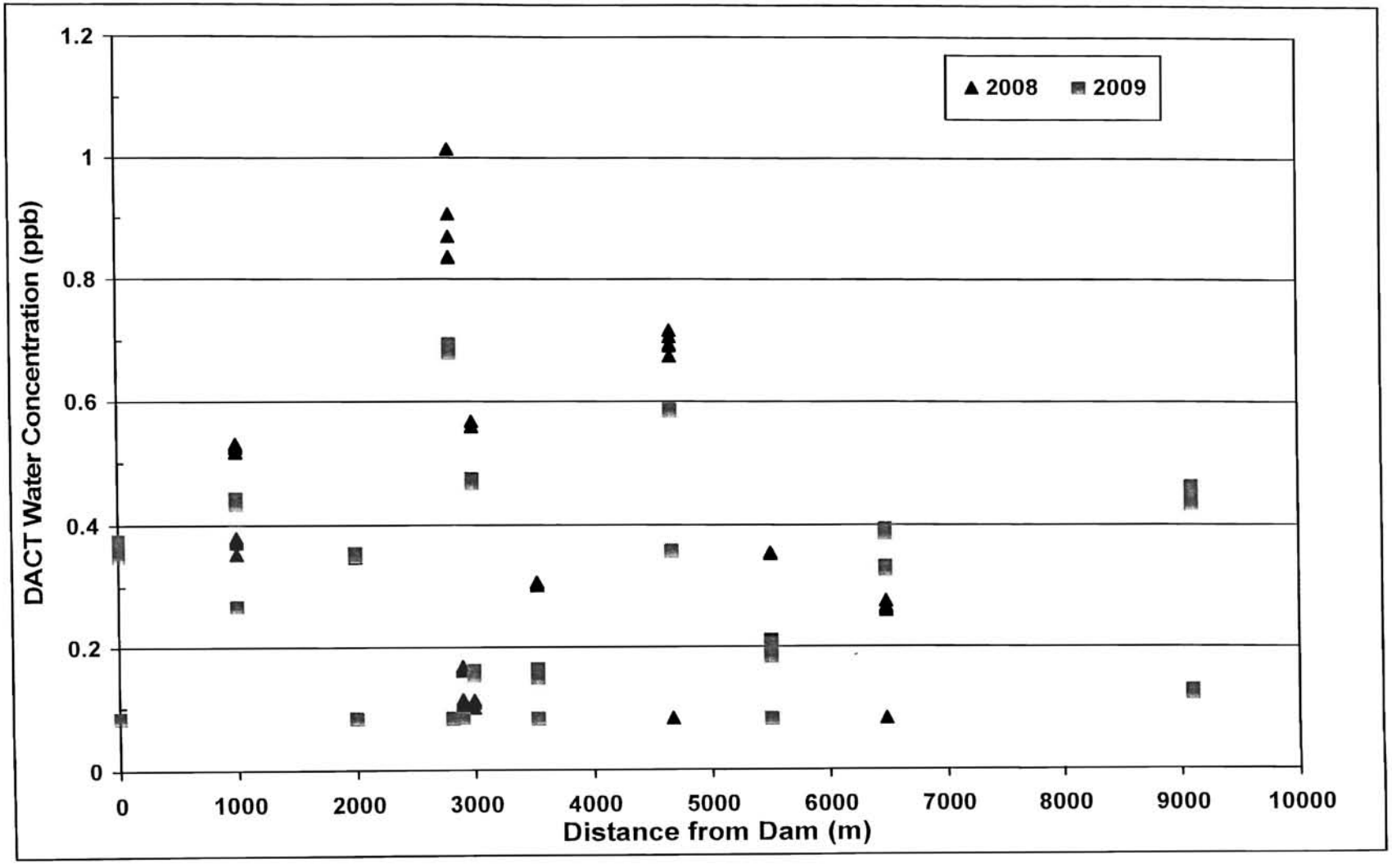

Figure 14. Concentrations of DACT found in water samples from the Embarras River, Illinois, in 2008 and 2009.

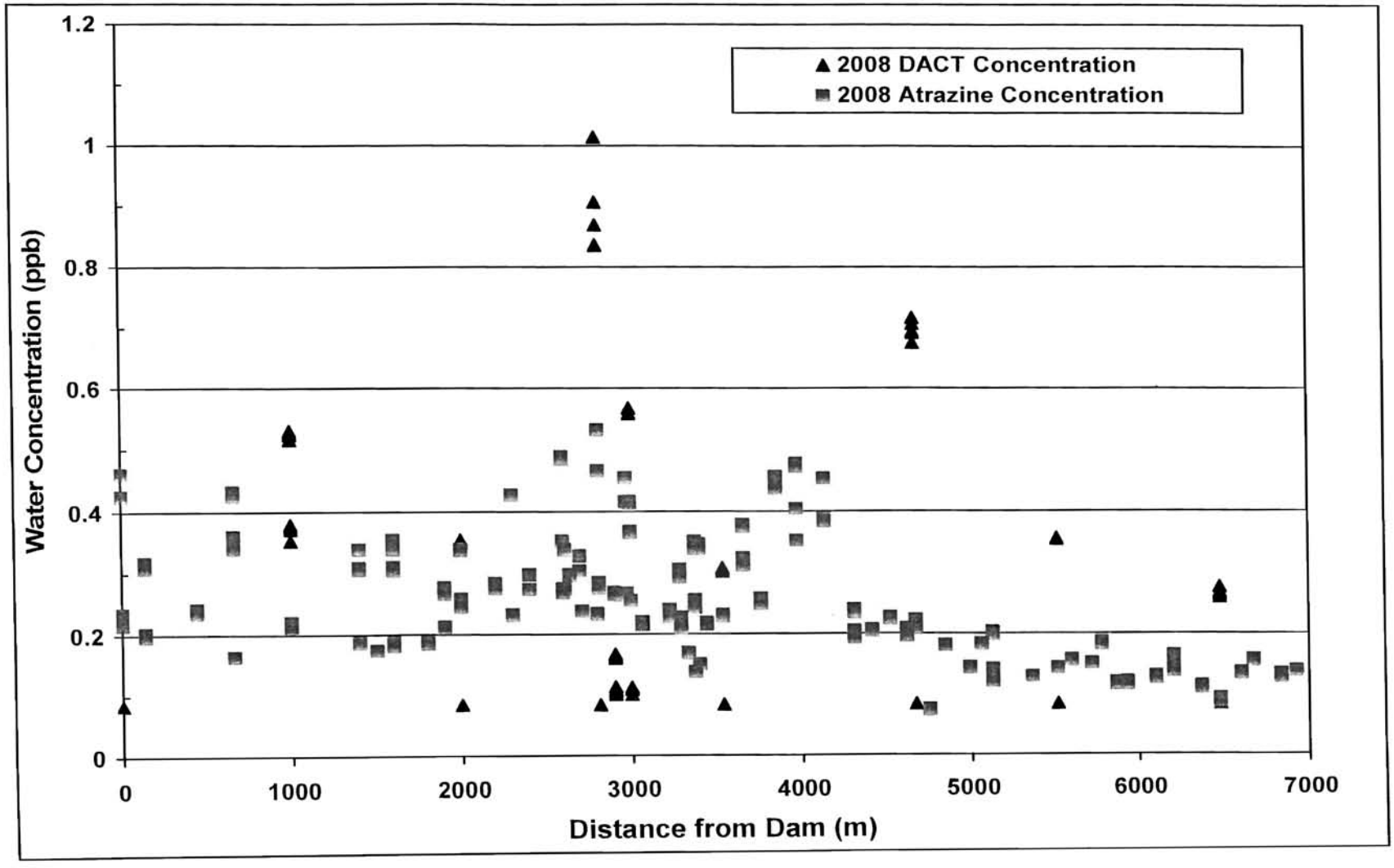

Figure 15. DACT and atrazine concentrations found in water samples from the Embarras River, Illinois, in 2008.

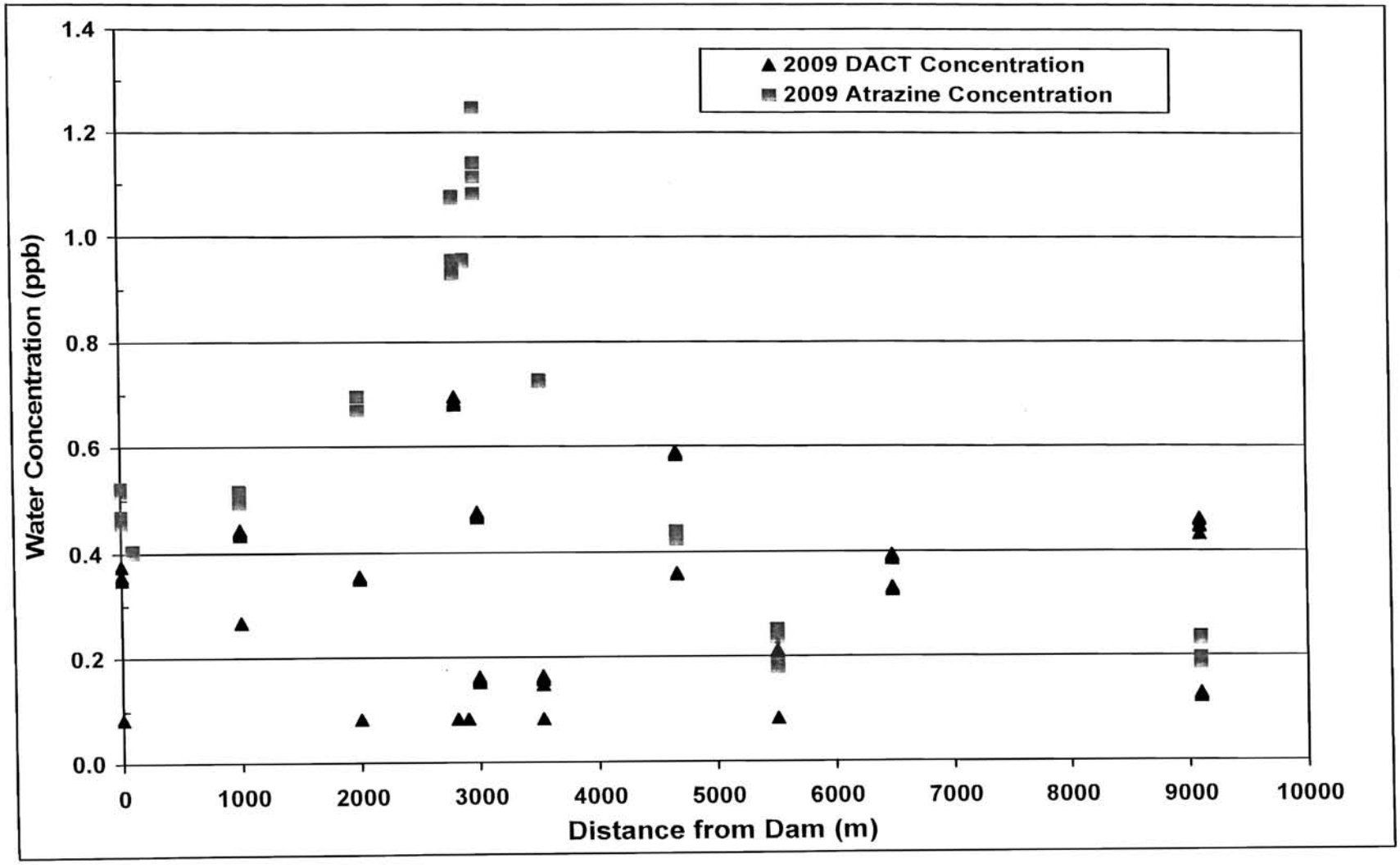

Figure 16. DACT and atrazine concentrations found in water samples from the Embarras River, Illinois, in 2009.

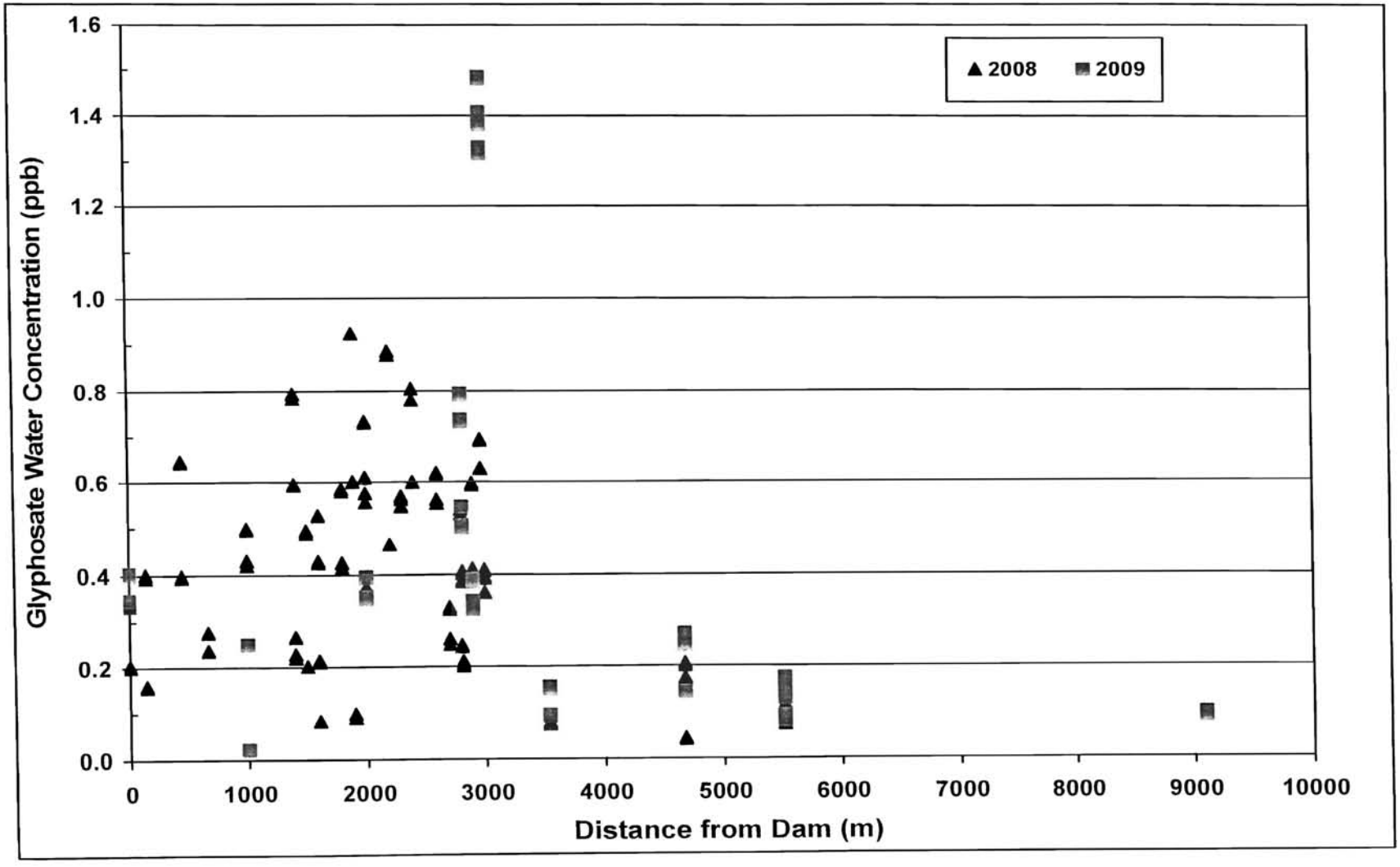

Figure 17. Concentrations of glyphosate found in water samples from the Embarras River, Illinois, in 2008 and 2009.

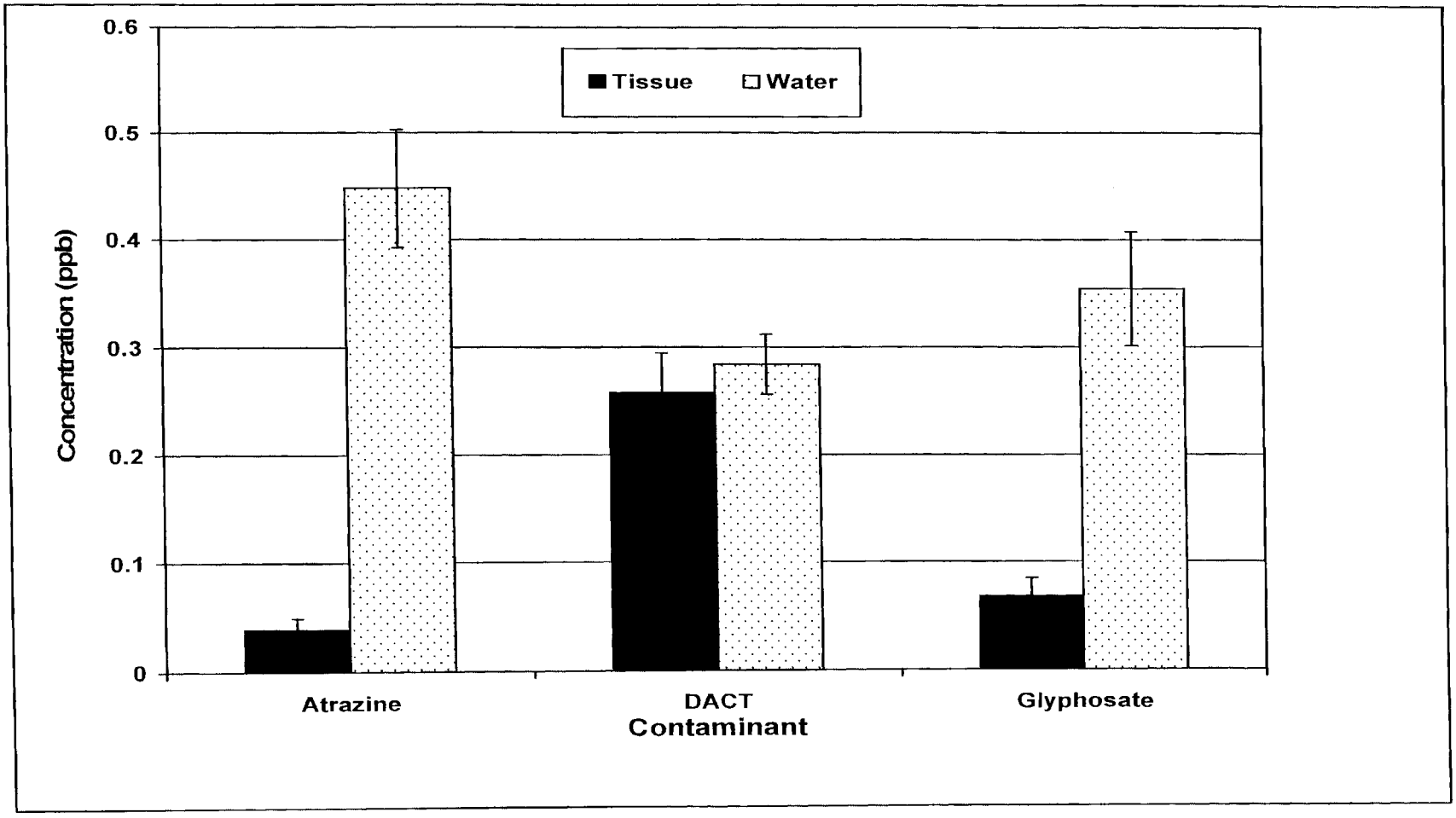

Figure 18. Mean concentrations of atrazine, DACT, and glyphosate in common snapping turtle tissue compared to water column concentrations at either the same or closest upstream site.

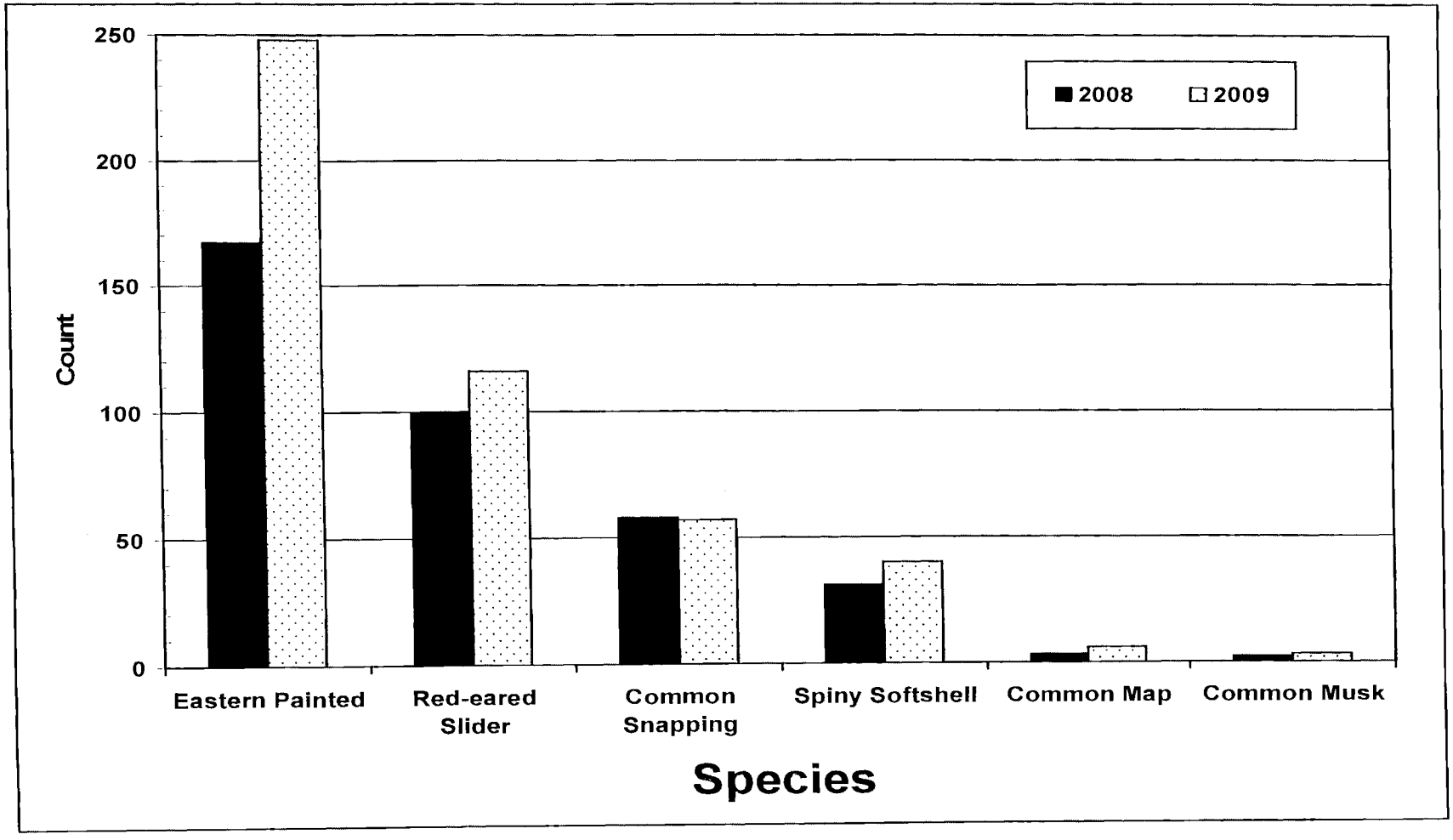

Figure 19. Relative abundance of turtle species captured in the Embarras River, Illinois, in 2008 and 2009. Count represents total captures and recaptures for snapping turtles and total captures for all other species.

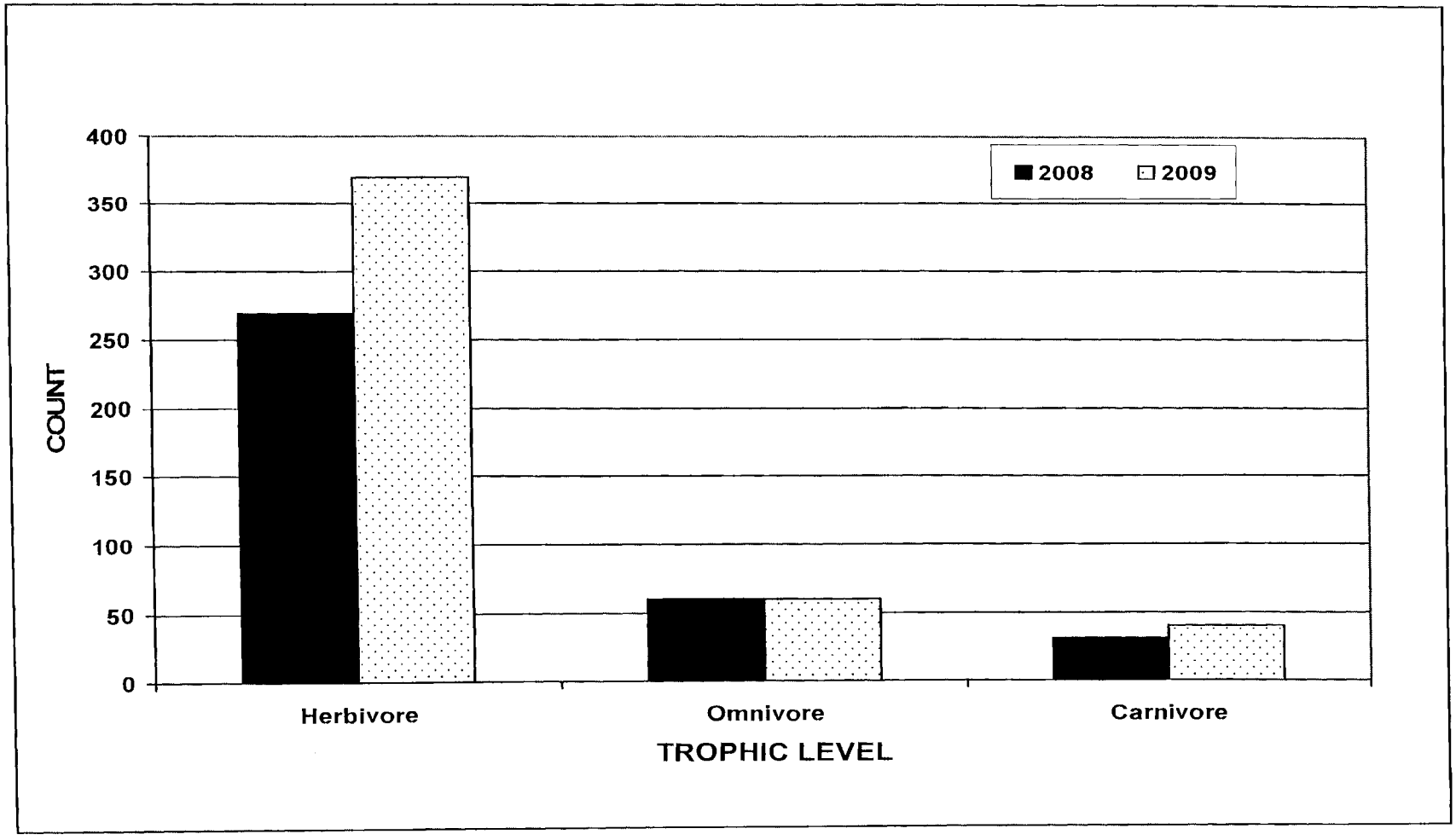

Figure 20. Relative abundance of turtles captured in the Embarras River, Illinois, in 2008 and 2009 by trophic level. Count represents total captures and recaptures for snapping turtles (omnivore) and total captures for all other species.

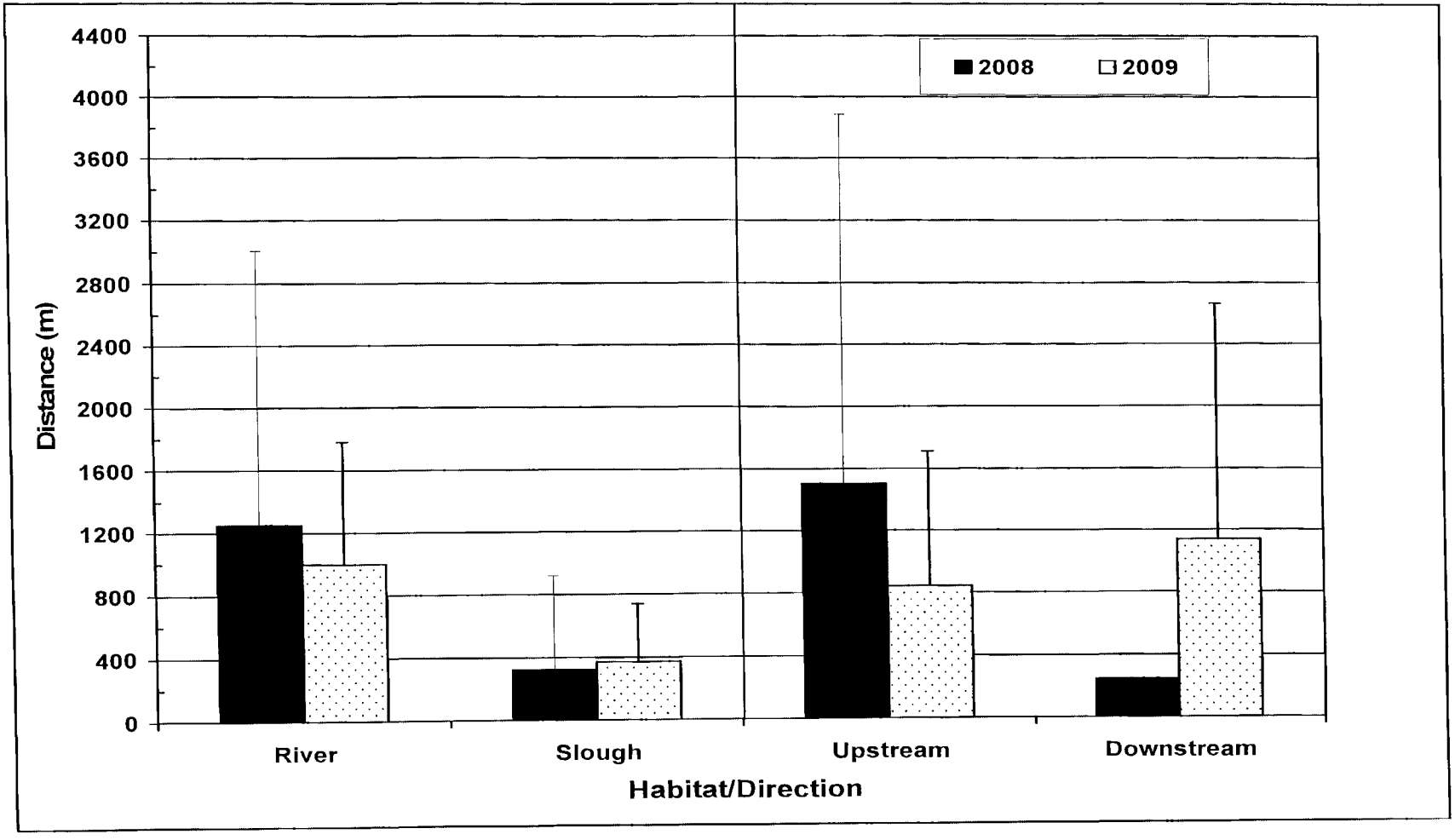

Figure 21. Movement distance of common snapping turtles in the river and slough habitats along with direction of movement in flowing water for each year trapped.

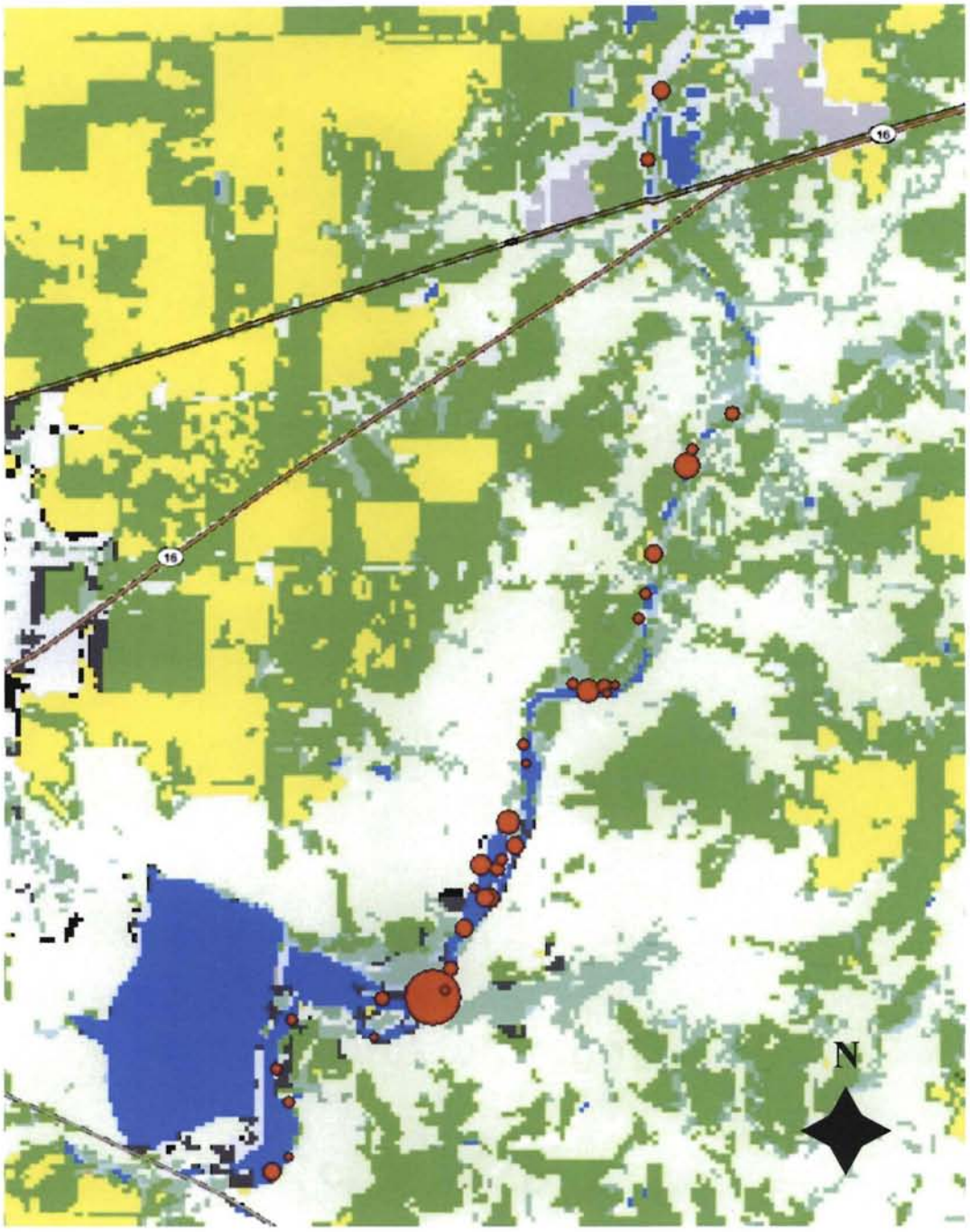

Figure 22. Location of turtle trapping sites in the Embarras River (Coles County, IL) and land usage along the river. Bright yellow represents corn and bright green represents soybean. The size of the symbol for trapping locations represent the atrazine concentration in turtle tissue for 2009 (larger circles are higher concentrations).